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CORRIGENDA IN VOL. 33

p. 138, line 5 *for* "solid" *read* vesicular.

p. 220. In the figure transpose *a* and *b*.

ORIGIN, NATURE AND BREEDING BEHAVIOUR
OF *OENOTHERA LAMARCKIANA* TRISOMICS

By D. G. CATCHESIDE

(University of London, King's College)

(With Seventeen Text-figures)

INTRODUCTION

HUGO DE VRIES AND K. BOEDIJN (1923, 1924) classified the numerous trisomics of *Oenothera Lamarckiana* into seven classes on the principle that there were seven haploid chromosomes (see also de Vries and Gates, 1928, for a more recent account). When the complex interchange system existing in this species was recognised, it was seen that it possessed more than seven different chromosomes. Thus Hoeppener and Renner (1929, p. 39) showed that there should be thirteen different trisomics, one corresponding to each of the twelve chromosomes in the ring and one to the free pair. These trisomics, styled by de Vries "dimorphic", regularly segregate normal diploid *Oe. Lamarckiana* on selfing, or pollination by *Oe. Lamarckiana* if the pollen is sterile as in *lata*. They behave substantially like the primary trisomics of other, structurally homozygous, plants such as *Datura stramonium*. Their cytology has become fairly well known, chiefly through the investigations of Håkansson (1926, 1930).

Further, it has been shown (Catcheside, 1934) that one of the frequent types of non-disjunction in the ring, namely double non-disjunction on the same side, should produce another trisomic type with the unique property of breeding true on selfing. These may be styled "monomorphic", in contrast to those which segregate *Oe. Lamarckiana* on selfing. Several *Oenothera* trisomics of this nature are well known, *oblonga* and *albida*, two of the original types described by de Vries, having this curious property. The behaviour of their chromosomes at meiosis is unfortunately very imperfectly known. Håkansson (1930) has described the chromosome behaviour in *curta*, another trisomic which breeds true on selfing; his observations are in accord with the theoretical requirements (Catcheside, 1934).

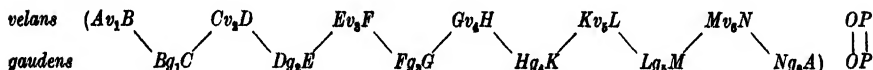
The number of monomorphic trisomics, as well as their cytological features, have been worked out by Mr C. E. Ford (unpublished), who

has shown that a possible forty-eight may result from the functioning of gametes produced by double non-disjunction on the same side.

I shall show that there are a further twelve monomorphic trisomics, de Vries's accessory trisomics, one corresponding to each of the twelve dimorphic trisomics having an additional ring chromosome. The present paper is an attempt to infer from the assumption of the complex heterozygote (Renner, 1917, 1925) and segmental interchange (Darlington, 1929, 1931) hypotheses the theoretical cytological and genetical behaviour of *Oe. Lamarckiana* trisomics. Actual genetical and cytological findings will be compared with the deductions.

DISJUNCTION AND NON-DISJUNCTION IN *OENOTHERA LAMARCKIANA*

Cytogenetic investigations have shown that *Oe. Lamarckiana* is a complex interchange heterozygote, regularly producing two gametic types, the Renner complexes *velans* and *gaudens*. We may designate the chromosomes, arranged as a ring of twelve and a free pair at meiosis, thus:

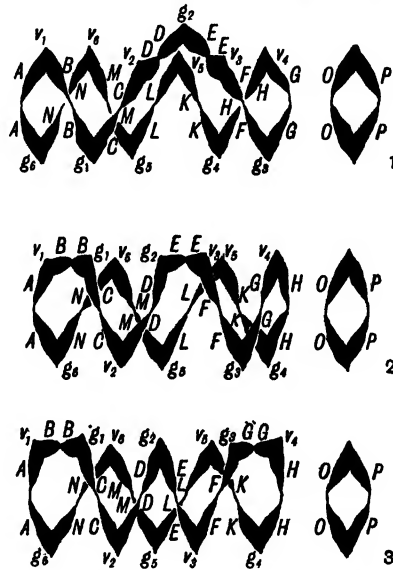


So far as the differences may be defined, each chromosome in the ring consists of three segments, two distal pairing segments and a proximal non-pairing differential segment (Darlington, 1931). The two chromosomes in the free pair have two pairing segments and no differential. The fourteen pairing segments are labelled A to P, the six *velans* differentials v₁ to v₆, and the six *gaudens* differentials g₁ to g₆.

Nothing is known of the relative lengths of the pairing and differential regions of the chromosomes, nor whether every chromosome in the ring has a differential segment. The data, however, point to the conclusions that (1) viability of gametes is conditional upon the presence of at least one of each of the fourteen pairing segments, and (2) viability of zygotes depends upon the presence of at least two of each of the pairing segments. If every ring chromosome has a differential segment, it is clear that some of these may be omitted without prejudice to the plant lacking them. For example, the half-mutant *rubrinervis* (*subvelans. paenevelans*) would lack three *gaudens* differentials, while the full mutant *deserens* (*subvelans. subvelans*) would lack three *gaudens* and three *velans* differential segments. In the following, it is assumed that the pairing segments are essential, and that some at least of the differentials are non-essential.

A consideration of the non-disjunctional anaphase arrangements,

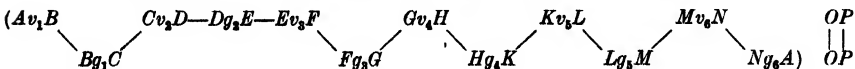
found in *Oe. Lamarckiana* and other *Oenothera* species, shows that only two kinds are capable of producing viable gametes, that is gametes lacking no pairing segments. In each case eight-chromosome gametes would be produced, and with normal *velans*, or *gaudens*, gametes these should yield fifteen-chromosome zygotes, having a full complement of



Figs. 1-3. Diagrams of non-disjunction in *Oenothera Lamarckiana* capable of yielding viable gametes.

pairing segments. Provided that the differential segments did not introduce a lethal balance, these fifteen-chromosome zygotes would grow into trisomic plants.

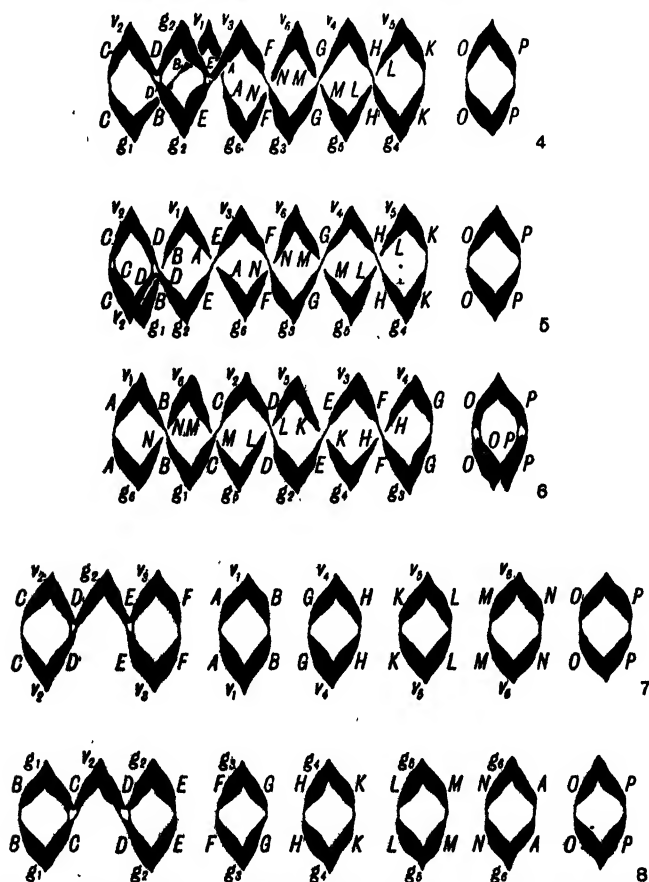
In simple non-disjunction, three adjacent chromosomes (cf. Fig. 1) in the ring may pass to the same pole at anaphase I, thus:



The eight-chromosome gametes produced in this way would carry all the *velans* chromosomes together with one whole *gaudens* chromosome; for convenience we may write it *velans* + Dg_2E . It may mate either with a *gaudens* or a *velans* gamete, producing respectively *gaudens.velans* + Dg_2E or *velans.velans* + Dg_2E zygotes (Figs. 4 and 7). The former represents the constitution of the well-known dimorphic trisomics of de Vries, such as *lata*, *scintillans*, etc., since it has the capacity of segre-

gating *Lamareckiana* (*velans.gaudens*) on selfing. With regard to the latter combination, it is well known that *velans.velans* is a lethal combination; but it is conceivable that *Dg₂E* could add to *velans.velans* enough to render the fifteen-chromosome zygote viable, though the plant produced might be more or less weakly. It would breed true on selfing because every other combination it could produce would be lethal; for convenience of reference they may be called monomorphic-I trisomics. It is probable that *oblonga* and *albida* have this type of constitution.

The constitutions of the corresponding trisomics produced from an eight-chromosome gamete carrying *gaudens* and one of the *velans* chromosomes are shown diagrammatically in Figs. 5 and 8.

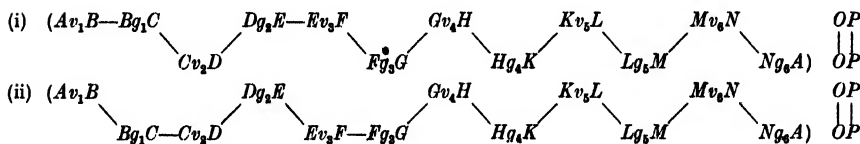


Figs. 4-8. Diagrams of *Oenothera Lamareckiana* trisomics. Figs. 4-6 show dimorphic trisomics; Figs. 7 and 8 monomorphic-I trisomics corresponding to the dimorphics in Figs. 4 and 5 respectively.

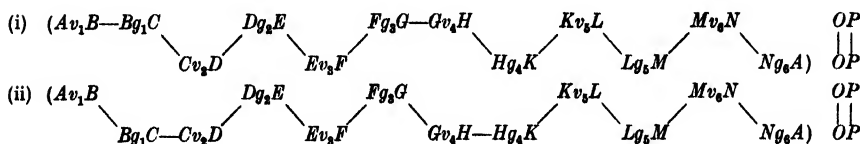
There are twelve different eight-chromosome gametes possible, each involving a different ring chromosome; therefore we may expect twelve dimorphic trisomics and twelve monomorphic-I trisomics, one of each corresponding to each of the twelve chromosomes in the ring. In addition there should be a thirteenth dimorphic trisomic corresponding to the free pair (Fig. 6).

Double non-disjunction on the same side would produce eight-chromosome gametes having one each of twelve distal pairing segments and two each of the remaining two pairing segments. If the vital balance were not disturbed by the differential segments present, the gametes could function to produce monomorphic trisomics. These, on selfing, would produce two gametic types of a constitution such that the only viable combination repeats the trisomic zygote; thus they would breed true owing to the elimination of the other possible combinations.

In double non-disjunction on the same side, two adjacent chromosomes at two different places around the ring pass to the same pole; these two groups of non-disjunctional chromosomes would be separated by odd numbers of chromosomes. In the case of a ring of twelve chromosomes (as shown by Ford (unpublished)) there are two cytologically distinguishable positions: (A) in which the double non-disjunctions are separated by one and seven chromosomes respectively, *e.g.*

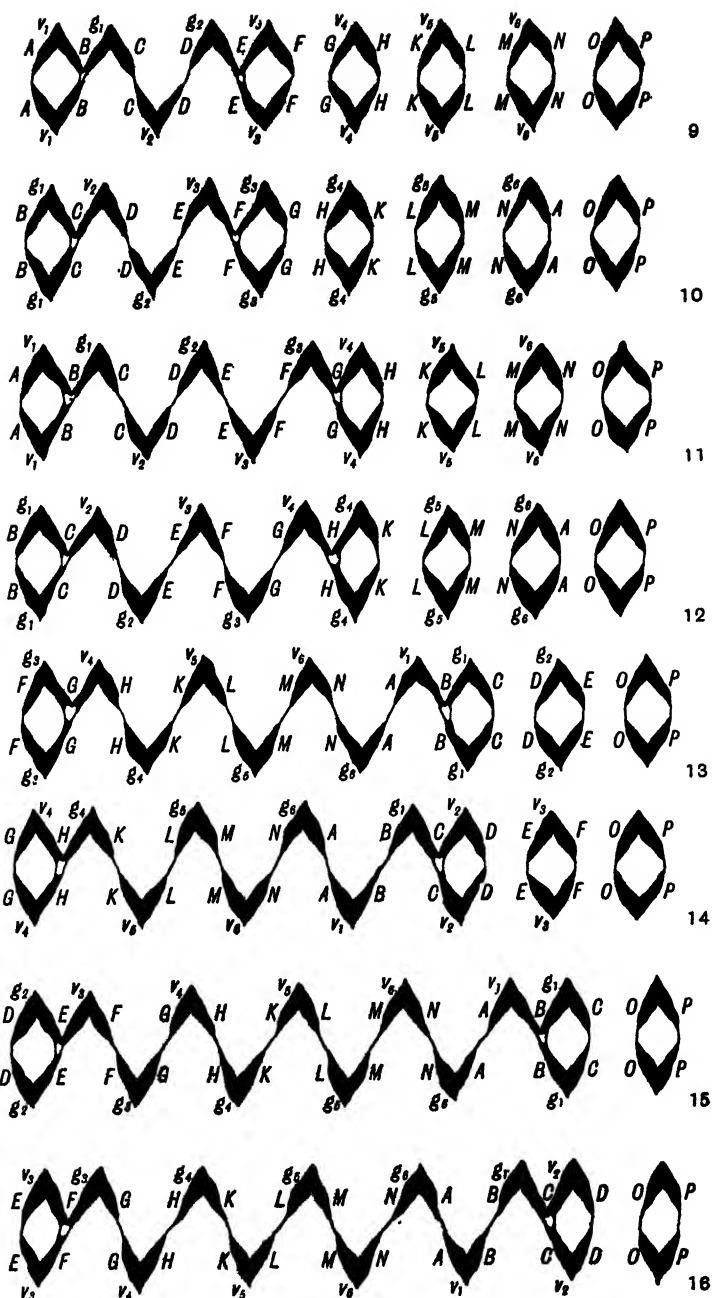


and (B) in which the double non-disjunctions are separated by three and five chromosomes respectively, *e.g.*



In each type, the double non-disjunctions may occupy any one of twelve positions in the ring, and each eight-chromosome gamete may be combined with either a *velans* or a *gaudens* gamete. Thus a possible total of forty-eight monomorphic trisomics may result from double non-disjunction on the same side in the ring of twelve of *Oe. Lamareckiana*.

These forty-eight trisomics would belong to four cytologically dis-



Figs. 9-16. Diagrams of *Oenothera Lamarckiana* trisomics. Figs. 9 and 10, monomorphic-II; Figs. 11 and 12, monomorphic-III; Figs. 13 and 14, monomorphic-IV; Figs. 15 and 16, monomorphic-V.

tinguishable classes, each containing twelve different mutant types. The four classes are, using the examples quoted above:

(1) Two pairs connected by a chain of three chromosomes, together with four free pairs, *e.g.* A (i) with *velans* (Fig. 9) and A (ii) with *gaudens* (Fig. 10) (monomorphic-II).

(2) Two pairs connected by a chain of five chromosomes, together with three free pairs, *e.g.* B (i) with *velans* (Fig. 11) and B (ii) with *gaudens* (Fig. 12) (monomorphic-III).

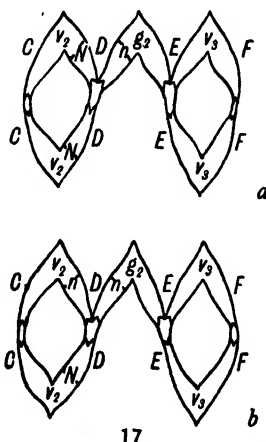


Fig. 17. Diagrams of the association of five in *Oenothera oblonga*:
(a) NNn; (b) Nnn. For explanation see text.

(3) Two pairs connected by a chain of seven chromosomes, together with two free pairs, *e.g.* B (i) with *gaudens* (Fig. 13) and B (ii) with *velans* (Fig. 14) (monomorphic-IV).

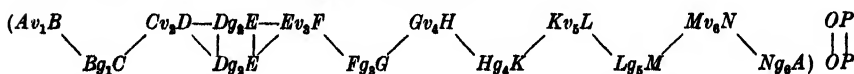
(4) Two pairs connected by a chain of nine chromosomes, together with one free pair, *e.g.* A (i) with *gaudens* (Fig. 15) and A (ii) with *velans* (Fig. 16) (monomorphic-V).

In each class, six members have *velans* as the second gamete, the other six having *gaudens*. They are those which have been called accessory and secondary trisomics by de Vries.

In all, neglecting considerations of inviability, there is therefore a maximum possible 73 trisomics, 13 being dimorphic and 60 monomorphic. They may all originate directly from *Oe. Lamarckiana*, none involve structural changes in the chromosomes and therefore they are primary trisomics in the strict sense.

BREEDING BEHAVIOUR OF DIMORPHIC TRISOMICS

The trisomic in which the extra chromosome corresponds to the free pair can yield, as viable zygotes, on selfing, only *Lamarckiana* (*velans.gaudens*) and the trisomic (*velans.gaudens* + *O.P.*). Those in which the extra chromosome is a member of the ring of twelve may produce further viable zygotes. In a dimorphic trisomic of the constitution *velans.gaudens* + Dg_2E (Fig. 1), i.e.



the effective gametic output on the female side, assuming no gametic elimination, would be *velans*, *gaudens*, *velans* + Dg_2E and *gaudens* + Dg_2E in equal numbers, and on the male side *velans* and *gaudens* in equal numbers. Eight-chromosome pollen grains function but rarely and may be neglected for our present purpose. The frequencies of the possible zygotic types, in the absence of gametic competition, amongst the pollen would be:

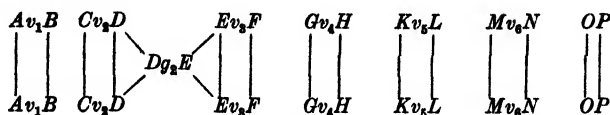
Constitution	Phenotype	Relative frequency
<i>velans.velans</i>	Lethal	1
<i>velans.gaudens</i>	<i>Lamarckiana</i>	2
<i>velans.velans</i> + Dg_2E	Monomorphic-I trisomic	1
<i>velans.gaudens</i> + Dg_2E	Dimorphic trisomic	2
<i>gaudens.gaudens</i>	Lethal	1
<i>gaudens.gaudens</i> + Dg_2E	Probably lethal	1

The nature and intensity of gametic elimination, which is likely to be different for each trisomic considered, must modify the proportions of the viable combinations in various ways. The most important feature is that on selfing a dimorphic trisomic, or pollinating it with *Oe. Lamarckiana*, a monomorphic-I trisomic should be obtained in the progeny, provided that the presence of the extra chromosome rendered the combination viable. This segregated trisomic (an accessory, according to the nomenclature of de Vries) would be identical with one of those derived directly from *Oe. Lamarckiana*. In the progeny of the selfed dimorphic trisomic, it should, in the absence of gametic competition, be half as frequent as the dimorphic ones.

BREEDING BEHAVIOUR OF MONOMORPHIC-I TRISOMICS

These trisomics have a characteristic chromosome configuration at meiosis, namely, two ring pairs joined by one chromosome together with five free pairs of chromosomes. The constitution of a typical one, viz.

velans.velans + Dg_2E derived from the dimorphic trisomic considered above, is as follows:



The effective gametic output is *velans* and *velans* + Dg_2E on the female side and *velans* only on the male side. The zygotic types on selfing are therefore *velans.velans* and *velans.velans* + Dg_2E ; the former is lethal and the latter repeats the monomorphic trisomic. It therefore breeds true owing to inviability of the only other possible combination.

When such a trisomic is pollinated by *Oe. Lamarckiana*, both *velans* and *gaudens* gametes are introduced on the male side, and the possible zygotic types are:

<i>velans.velans</i>	Lethal'
<i>velans.velans</i> + Dg_2E	Monomorphic-I trisomic
<i>velans.gaudens</i>	<i>Lamarckiana</i>
<i>velans.gaudens</i> + Dg_2E	Dimorphic trisomic

The relative frequencies clearly will depend upon the nature and intensity of any gametic competition that is involved.

We may now consider in how far these conclusions are supported in the literature of trisomic *Oe. Lamarckiana*.

Oenothera scintillans and *Oe. oblonga*

De Vries (1909a) early showed that *Oe. scintillans*, later found to be trisomic, regularly segregated a large proportion of *oblonga*, in addition to *Lamarckiana* and *scintillans*, on selfing. Clearly *scintillans* is a dimorphic trisomic in our present sense, while *oblonga*, which breeds true on selfing, is a monomorphic-I trisomic. According to de Vries (1913, 1919), *oblonga* has the constitution (haplo-*oblonga* + *velans*) × (*velans*). This means, on our hypothesis, that *oblonga* is *velans.velans* plus one *gaudens* chromosome (say Dg_2E), and that *scintillans* is *velans.gaudens* plus the same *gaudens* chromosome (Dg_2E).

In Table I are data taken from de Vries (1913, pp. 257-60); he had given the results as percentages, but they are here recalculated as actual numbers of individuals, since the figures are then less misleading. Altogether, *scintillans* selfed gave 830 *Lamarckiana*, 261 *scintillans* and 153 *oblonga*; clearly *oblonga* is only about half as frequent as *scintillans* in these progenies. De Vries (1925) has given further counts, based on a total of 7380 plants, which show substantially similar proportions.

The last two crosses in Table I, namely *scintillans* × *rubrinervis* (pollen *subvelans* and *paenevelans*) and *scintillans* × *oblonga* (pollen *velans*) indicate that (*gaudens* + *Dg₂E*) gametes are rare among the ovules. De Vries (*Mutation Theory*, vol. 1) has also shown that the method of cultivation profoundly influences the frequencies of the different types in progenies of *Oe. scintillans*; evidently culture, especially weakness or strength of the individual plant, in some way can greatly modify the intensity of gametic competition.

TABLE I
Genetics of Oenothera scintillans

Parentage of family	Year	Numbers of individuals in family			
		<i>Lamarckiana</i>	<i>Scintillans</i>	<i>Oblonga</i>	Others
<i>Scintillans</i> self	1905	398	106	56	0
" "	1908	134	25	31	6
" "	1908	92	11	33	3
" "	1909, no. 1	107	50	20	2 <i>lata</i>
" "	1909, no. 2	84	63	13	3 <i>lata</i>
" "	1910, no. 1	15	6	0	0
<i>Scintillans</i> × <i>Lamarckiana</i>	1898	106	43	11	0
<i>Scintillans</i> × <i>Lamarckiana</i>	1908	220	26	47	0
<i>Scintillans</i> × <i>nanella</i>	1897	14	12	4	22 <i>nanella</i>
<i>Scintillans</i> × <i>nanella</i>	1908	34	6	8	72 <i>nanella</i>
<i>Scintillans</i> × <i>nanella</i>	1908	29	49	32	178 <i>nanella</i>
<i>Scintillans</i> × <i>rubrinervis</i>	1908	47	1	9	6 <i>subrobusta</i>
<i>Scintillans</i> × <i>oblonga</i>	1908	51	1	11	0

In Table II are summarised data bearing on the genetics of *Oe. oblonga*; the figures are taken from de Vries (1913, pp. 265-6), and again the percentages he has given are converted into actual numbers. In the three crosses in which *oblonga* is the female parent, and *Lamarckiana*, *nanella* and *scintillans* respectively the male parents, we should have

TABLE II
Genetics of Oenothera oblonga

Parentage of family	Year	Numbers of individuals in family		
		<i>Lamarckiana</i>	<i>Oblonga</i>	Others
<i>oblonga</i> × <i>Lamarckiana</i>	1907	108	5	3
<i>oblonga</i> × <i>Lamarckiana</i>	1908	46	2	0
<i>oblonga</i> × <i>Lamarckiana</i>	1911	69	11	0
<i>oblonga</i> × <i>nanella</i>	1908	32	6	2
<i>oblonga</i> × <i>nanella</i>	1911	18	60	52 <i>oblonga nanella</i>
				1 <i>albida</i>
<i>oblonga</i> × <i>scintillans</i>	1910	60	13	2 <i>lata</i>
<i>Lamarckiana</i> × <i>oblonga</i>	1908	70	0	0
<i>nanella</i> × <i>oblonga</i>	1908	40	0	0
<i>lata</i> × <i>oblonga</i>	1908	15	0	0
<i>scintillans</i> × <i>oblonga</i>	1908	52	11	0

expected on our hypothesis to find a proportion of *scintillans* among the progeny. This in fact was not observed. Either the hypothesis is wrong or the *scintillans* plants, which in any event would have been few in number, were overlooked. I am inclined to the latter view, since the data were obtained by sowing the seeds in pans or boxes and scoring the plants in the seedling stage. In these circumstances it would have been very easy to miss a few *scintillans* plants unless they were sought for specially. In the case of *scintillans* \times *oblonga*, also, there should have been some *scintillans* in the progeny unless the *gaudens* + Dg_2E gametes were eliminated from the ovules of *scintillans* through competition with *velans*. This means that the ovules of *scintillans* would carry only *velans*, *gaudens* and *velans* + Dg_2E , and that the proportion of *scintillans* and of *oblonga* in its progeny would depend upon the proportions of *velans* and *gaudens* pollen effecting fertilisation. This conclusion is supported by crosses between *scintillans* and other species (de Vries, 1913, 1925); *gaudens* + Dg_2E rarely or never functions.

The cytological evidence for the nature of *Oe. scintillans* and *Oe. oblonga* is far weaker than the genetical. Meiosis in *Oe. scintillans* has not been properly examined so far as I am aware.

Cleland (1923) states that in *Oe. oblonga* there may be a chain of three chromosomes and six ring pairs, a ring of five and five ring pairs, a chain of seven and four ring pairs or a chain of nine and three ring pairs. Evidently *oblonga* has an association of five chromosomes of some type, together with a number of ring pairs of chromosomes. The type of variation recorded is anomalous.¹

The relationship of other regular derivatives of *Oe. scintillans*, such as *Oe. persicaria* and *Oe. lancifolia* (de Vries and Gates, 1928), to the parental form and to *Oe. oblonga* is not yet known. Their elucidation will depend upon a repetition of the breeding experiments with a careful cytological control.

Oenothera lata and *Oe. albida*

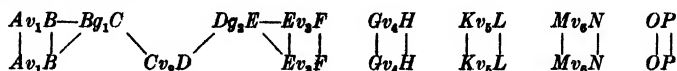
These are respectively a dimorphic and a monomorphic-I trisomic, comparable with the pair of trisomics *scintillans* and *oblonga*. The gametic constitution of *albida* (de Vries, 1923), namely (*albida* + *velutina*) \times (*velutina*), shows that the extra chromosome in *lata* is a *gaudens* chromosome,

¹ Further information from Prof. Cleland (*in litt.*) shows definitely that his *oblonga* arose from segmental interchange between corresponding segments of opposite complexes (Cf. Darlington 1931, p. 446-7). It is a tertiary trisomic and therefore not relevant to the present discussion.

say Lg_5M . *Lata* is therefore *velans.gaudens* + Lg_5M , while *albida* is *velans.velans* + Lg_5M . According to data reported by de Vries (1909 b), crosses with other species (*Cockerelli*, *biennis Chicago* and *Hookeri*) show that the female gametes in *lata* are chiefly *velans*, *gaudens* and *velans* + Lg_5M ; there are only a few *gaudens* + Lg_5M gametes. In a total count of 1802 individuals from *Oe. lata* × *Oe. Lamarckiana*, de Vries (1925) found 69 per cent. *Lamarckiana*, 19 per cent. *lata*, 9 per cent. *albida* and 3 per cent. mutants. Here again, in accordance with expectation, the *albida* trisomics are about half as frequent as the *lata* trisomics. Further, an F_1 family from *albida* × *Lamarckiana*, described by de Vries (1923), consisted of 60 individuals in the proportions, 47 per cent. *Lamarckiana*, 38 per cent. *albida*, and 9 per cent. *lata*. Most notable is the occurrence of the expected *lata* individuals in significant numbers. The cytological observations on meiosis in *lata*, especially those of Håkansson (1930) and Catchside (unpublished), agree with the requirements of the present hypothesis; the diagram in Fig. 4 correctly represents (apart from the lettering) the maximum configuration in *Oe. lata*. The chromosome behaviour at meiosis in *Oe. albida* is unknown.

BREEDING BEHAVIOUR OF MONOMORPHIC-II, III, IV AND V TRISOMICS

The properties of these forms will be fully understood if one typical example is expounded in entirety. Let us consider the monomorphic-II *velans* trisomic shown diagrammatically in Fig. 9; it consists of the eight-chromosome complex and *velans*. The constitutions of the chromosomes are therefore:

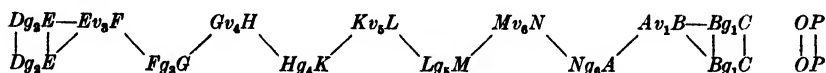


The eight-chromosome complex is essentially a *velans*, in which Cv_2D is replaced by Bg_1C and Dg_2E ; the lack of the differential v_2 is compensated by extra B and E pairing segments and the differentials g_1 and g_2 .

The fifteen-chromosome zygote is a *velans.velans*, lacking one Cv_2D chromosome, the absence of which is compensated by the two *gaudens* chromosomes Bg_1C and Dg_2E . The viability of the combination depends upon whether the two *gaudens* chromosomes can restore the balance so seriously disturbed by a preponderance of *velans* chromosomes. Let us assume that it is viable, and for simplicity let the eight-chromosome complex be referred to briefly as *Tc* (trisomic complex).

The effective gametic output on the female side is *Tc* and *velans*, and

on the male side *velans* only; the only viable zygotic type is *Tc.velans*, the trisomic. The plant therefore breeds true on selfing. When such a trisomic is pollinated by *Oe. Lamarckiana*, that is when the pollen consists of both *velans* and *gaudens*, the possible zygotic types are *velans.velans* (lethal), *velans.gaudens* (*Lamarckiana*), *velans.Tc* (monomorphic-II *velans* trisomic) and *gaudens.Tc*. It is conceivable that in some cases the last-named combination may also be viable. It would be a monomorphic trisomic belonging to a different type, namely, type V. Its chromosomal constitution would be:



It would be distinguishable phenotypically and cytologically from its maternal parent. It would breed true, and on pollinating with *Lamarckiana* would segregate its maternal parent, as well as *Lamarckiana* and itself. Clearly, these monomorphic trisomics are related in pairs as follows:

- (a) Monomorphic-II (*velans* pollen) with monomorphic-V (*gaudens* pollen).
- (b) Monomorphic-II (*gaudens* pollen) with monomorphic-V (*velans* pollen).
- (c) Monomorphic-III (*velans* pollen) with monomorphic-IV (*gaudens* pollen).
- (d) Monomorphic-III (*gaudens* pollen) with monomorphic-IV (*velans* pollen).

Twenty-four different eight-chromosome gametes may be combined either with *velans* or *gaudens* to give forty-eight fifteen-chromosome zygotic types. Two related monomorphic trisomics crossed with a different homozygous form (e.g. *blandina* or *Hookeri*) would yield identical trisomics; one would also give a *velutina* (*velans*), the other a *laeta* (*gaudens*) hybrid.

GENE CONSTITUTIONS OF *OENOTHERA LAMARCKIANA* TRISOMICS

The genotypes of different trisomics in respect of genes situated in the ring of twelve and normally heterozygous in the diploid may yield evidence leading to the location of the genes in particular chromosomes. Let us take the general case of the allelomorphs **A** in *velans* and **a** in *gaudens*. One of the dimorphic trisomics would be **Aaa**, a second **AAa** and the remaining eleven **Aa**. Ten of the latter would be those which

have an extra ring chromosome, the eleventh that which has an extra ring pair chromosome. The frequencies of the different constitutions in the various monomorphic trisomics are enumerated in Table III.

TABLE III
Numbers of trisomics with different genic constitutions
(*A velans*.*a gaudens*)

Genic constitutions	AAa	Aaa	AA	Aa	aa
Dimorphic	1	1	—	10 + 1	—
Monomorphic-I <i>velans</i> pollen	1	—	5	—	—
Monomorphic-I <i>gaudens</i> pollen	—	1	—	—	5
Monomorphic-II <i>velans</i> pollen	1	—	4	1	—
Monomorphic-II <i>gaudens</i> pollen	—	1	—	1	4
Monomorphic-III <i>velans</i> pollen	1	—	3	2	—
Monomorphic-III <i>gaudens</i> pollen	—	1	—	2	3
Monomorphic-IV <i>velans</i> pollen	1	—	2	3	—
Monomorphic-IV <i>gaudens</i> pollen	—	1	—	3	2
Monomorphic-V <i>velans</i> pollen	1	—	1	4	—
Monomorphic-V <i>gaudens</i> pollen	—	1	—	4	1

Recessive homozygosis is easily observed; fifteen out of the sixty monomorphic trisomics would be homozygous for a particular recessive, which was heterozygous in normal diploid *Oe. Lamarckiana*. Five of these would be segregated by a dimorphic trisomic of the constitution **Aa**.

In terms of particular genes, if *Lamarckiana* is **N velans**.**n gaudens** (**N** tall, **n nanella** stature), five of the dimorphic trisomics having an extra *velans* chromosome and a genic constitution **Nn**, could yield a **nn** monomorphic-I trisomic, that is homozygous for *nanella* stature. It seems likely that the dimorphic trisomic *cana* described by de Vries (1916) is one of these five. In Table III of that paper, de Vries shows that in selfed progenies of *cana* totalling 610 individuals, there were 371 *Lamarckiana*, 206 *cana* and 33 *cana-nanella*. The last group, amounting to 5.4 per cent. of the total, had the stature of *nanella* with the marks of *cana*. Their frequency of occurrence was more than five times that of *nanella* in *Lamarckiana*. Were they trisomic? Unfortunately, there are no further data bearing upon this matter; de Vries does not appear to have grown any progeny from these *cana-nanella* plants.

One of the dimorphic trisomics, as we have seen, would be **Nnn**, when the extra *gaudens* chromosome was the one carrying **n**. The following genetical evidence indicates that *scintillans* is this trisomic. If this were so, *oblonga* would normally have the constitution **NNn** and be capable of yielding only **N velans** and **Nn oblonga** gametes in the absence of a cross-over. Occasionally, however, through a cross-over, **Nnn oblonga** plants could arise and would differ in their breeding be-

haviour. Data given by de Vries (1913) show that this has happened (see Table II). When *nanella* (n *velans*. n *gaudens*) was pollinated by *oblonga*, only tall (NN) *Lamarckiana* plants were obtained. The *velans* gametes of the 1908 *oblonga* were N as expected. *Oblonga* (1908) pollinated by *nanella* gave only tall plants, but *oblonga* (1911) pollinated by *nanella* gave about 40 per cent. dwarf plants. Evidently *oblonga* (1908) was NNn , while *oblonga* (1911) was Nnn . These two constitutions are shown diagrammatically in Fig. 17, and their genetic properties are compared below:

Constitution	NNn <i>oblonga</i>	Nnn <i>oblonga</i>
Ovule gametes	$\left\{ \begin{array}{l} N \text{ velans} \\ Nn \text{ (oblonga) gamete} \end{array} \right.$	$\left\{ \begin{array}{l} N \text{ velans} \\ n \text{ velans} \\ Nn \text{ (oblonga) gamete} \\ nn \text{ (oblonga) gamete} \end{array} \right.$
$\left\{ \begin{array}{l} \text{Viable zygotes} \\ \text{with nanella} \\ \text{(n velans.} \\ \text{n gaudens)} \\ \text{as pollen} \\ \text{parent} \end{array} \right.$	$\left\{ \begin{array}{l} Nnn \text{ oblonga} \\ Nn \text{ Lamarckiana} \\ Nnn \text{ scintillans} \end{array} \right.$	$\left\{ \begin{array}{l} Nnn \text{ oblonga} \\ nnn \text{ oblonga} \\ Nn \text{ Lamarckiana} \\ nn \text{ Lamarckiana} \\ Nnn \text{ scintillans} \\ nnn \text{ scintillans} \end{array} \right.$

Except for the absence of *scintillans*, the observations are in fair accord with the hypothesis. We may therefore conclude that the extra chromosome in *scintillans* is not only a *gaudens* chromosome, but is actually the one in which the factor n for *nanella* stature is located.

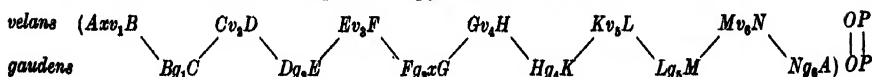
LETHAL GENES OR LETHAL BALANCE

If the inviability of homozygous zygotes is a consequence of the action of recessive lethal genes, and *Oe. Lamarckiana* is L_1L_2 *velans*. l_1L_2 *gaudens*, then five of the six *velans* monomorphic-I trisomics would be L_2L_2 and one $L_2l_2L_2$. Apparently, on the hypothesis of lethal genes, only one viable monomorphic-I *velans* trisomic should be obtainable; we know that there are at least two, viz. *oblonga* and *albida*. This throws doubt upon the hypothesis of specific lethal genes. It would appear rather that there is a vital chromosome balance involved, determined by the disposition of deficiencies and reduplications within the complexes.

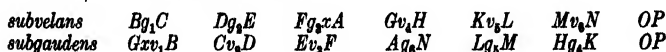
THE TRISOMICS OF *OENOTHERA RUBRINERVIS*

Oenothera rubrinervis is a half-mutant whose complexes are *paenevelans* and *subvelans*; *paenevelans* is essentially *velans* while *subvelans* is partly *velans* and partly *gaudens*, but with one interchanged *gaudens* chromosome. It is of interest to discover how many of the trisomics that *Oe. rubrinervis* could produce would be identical with those that *Oe. Lamarckiana* could produce.

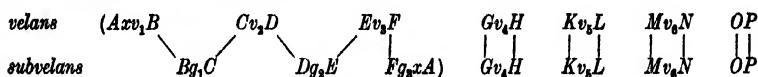
Using the nomenclature adopted by Darlington (1931), *subvelans* would be formed as a consequence of crossing-over in the interstitial segments x and x in Axv_1B and Fg_3xG , as follows:



The new interchange complexes would be



and *Oe. rubrinervis* would be



Oe. rubrinervis, with a ring of six chromosomes and four free pairs, could produce twenty-two different primary trisomics, of which only three would be identical with *Oe. Lamarckiana* primary trisomics, the other nineteen being more or less different. They are:

(1) Six dimorphic trisomics, one corresponding to each of the six ring-forming chromosomes; none would be identical with *Oe. Lamarckiana* dimorphics.

(2) Six corresponding monomorphic-I trisomics, of which two would be identical with *Oe. Lamarckiana* monomorphic-I trisomics, viz. *velans.velans* + Bg_1C and *velans.velans* + Dg_2E .

(3) Four dimorphic trisomics, one corresponding to each of the four pair-forming chromosomes; all would be different from any *Oe. Lamarckiana* trisomics.

(4) Three monomorphic-II trisomics, having *velans* as pollen; one would be identical with an *Oe. Lamarckiana* monomorphic-II trisomic, namely that having Axv_1B , Bg_1C , Dg_2E , Ev_3F , Gv_4H , Kv_5L , Mv_6N and OP as the eight-chromosome complex.

(5) Three monomorphic-II trisomics, having *subvelans* as pollen; all would be different from any *Oe. Lamarckiana* trisomics.

These conclusions are in harmony with the statement of de Vries (1919, p. 2) that "*Oenothera rubrinervis* is not known to possess any noticeable degree of mutability; it has, especially, never produced those mutants which are of so common occurrence in allied mutating forms." There is only a small chance that the three trisomics in common would be amongst the more frequent ones produced by *Oe. Lamarckiana*.

TRISOMIC RATIOS IN *OENOTHERA LAMARCKIANA*

Genetic ratios in *Oe. Lamarckiana* are complicated by the complex interchange genetic situation. Homozygotes are segregated only through crossing-over of the genes concerned from one complex to the other. The frequency of homozygosis of recessives, in the progeny of a selfed heterozygote, is therefore a measure of the cross-over value between the complex differentials and particular gene loci. It is necessary to determine the relationship for different cases. Cleland and Brittingham (1934) have calculated, for particular cross-over values, the frequencies of recessives obtainable on selfing heterozygous individuals of isogamous species such as *Oe. Lamarckiana*. The following is a rather fuller treatment of the same problem.

(i) Ratios from **Aa** *Lamarckiana*

Let it be supposed that the constitution is **A** *velans*. **a** *gaudens*. This will be written **Av**. **ag**, and similarly for other constitutions. Let crossing-over of **A** and **a** between the complexes occur in a fraction p of meioses both of pollen mother cells and megaspore mother cells. The proportion of effective gametes would therefore be

$$(2-p) \text{Av} : p \text{av} : (2-p) \text{ag} : p \text{Ag},$$

giving a cross-over value of $50p$. The proportions of viable zygotes (amounting to half the total) on selfing would be

$$(4-4p+p^2) \text{Av}.\text{ag} : (2p-p^2) \text{Av}.\text{ag} : p^2 \text{av}.\text{Ag} : (2p-p^2) \text{av}.\text{ag}.$$

The phenotypic ratio on selfing would thus be

$$(4-2p+p^2) \text{A} : (2p-p^2) \text{a},$$

and the observed frequency of recessive homozygosis (x) in selfed populations of **Aa** *Lamarckiana* would be $\frac{1}{4}(2p-p^2)$. Values are given in Table IV, and from a knowledge of x the value of p can be found in any particular case.

A cross of **Aa** *Lamarckiana* with **aa** *Lamarckiana* would of course yield equal numbers of **A** *Lamarckiana* and **a** *Lamarckiana*. But a cross between **Aa** *Lamarckiana* and, say, **aa** *blandina* (or other suitable form) would yield progeny of four distinguishable types, viz. **A** *velans*. **a** ^h*blandina*, **a** *velans*. **a** ^h*blandina*, **A** *gaudens*. **a** ^h*blandina* and **a** *gaudens*. **a** ^h*blandina*, in proportions identical with the gametic ratios in the heterozygous parent. Further, such a cross would yield two independent determinations of the value of p , namely, (1) the ratio of the cross-over *velans* type

to the total *velans* type, and (2) the ratio of the cross-over *gaudens* type to the total *gaudens* type.

TABLE IV

Frequencies of recessive homozygosis for specific cross-over values on selfing Aa diploid Oenothera Lamarckiana and Aaa trisomic Oe. Lamarckiana

Meiotic cells with a cross-over between locus of A and the differential %	Values of recessive homozygosis on selfing (%)		
	Aa diploid	Aaa trisomic	
		Amongst <i>Lamarckiana</i> segregates	Amongst all segregates
1	0.5	0.32	0.27
2	0.99	0.66	0.58
3	1.48	0.99	0.87
4	1.96	1.32	1.16
5	2.44	1.96	1.45
6	2.91	1.96	1.74
7	3.38	2.28	2.03
8	3.84	2.60	2.32
9	4.30	2.91	2.61
10	4.75	3.22	2.90
20	9.0	6.22	5.75
30	12.75	9.0	8.56
40	16.0	11.56	11.33
50	18.75	13.9	14.06
60	21.0	16.0	16.75
70	22.75	17.89	19.4
80	24.0	20.67	22.0
90	24.75	21.0	24.56
100	25.0	22.22	27.08

(ii) *Ratios from Aaa dimorphic trisomic Lamarckiana*

Let it be supposed that the constitution is **A** *velans*. **a** *gaudens* together with the *gaudens* chromosome carrying **a**, and that the proportion of meiotic cells with a cross-over between the locus of **A** (or **a**) and the differentials is *p*. With random mating in pairs of the three identical parts, two-thirds of the meiotic cells would have **A** and **a** paired (the other **a** unpaired) and one-third would have **a** paired with **a** (the **A** unpaired). Cross-overs between **a** and **a**, of course, cannot be detected. The gametic ratios amongst the ovules, assuming that the extra chromosome is always included in one or other gamete and that there is no gametic competition (Renner effect), would be as follows:

$$(6-2p) \mathbf{Av} : 2p\mathbf{av} : (6-2p) \mathbf{ag} : 2p\mathbf{Ag} : (6-2p) (\mathbf{Av} + \mathbf{a}) : p (\mathbf{av} + \mathbf{a}) : p (\mathbf{av} + \mathbf{A}) : (6-2p) (\mathbf{ag} + \mathbf{a}) : p (\mathbf{ag} + \mathbf{A}) : p (\mathbf{Ag} + \mathbf{a}).$$

Amongst the pollen, assuming that the eight-chromosome gametes would be non-functional, the effective gametes would be in the ratios

$$(3-p) \mathbf{Av} : p\mathbf{av} : (3-p) \mathbf{ag} : p\mathbf{Ag}.$$

The zygotic proportions (neglecting inviable combinations) in the progeny of **Aaa** *Lamarckiana* selfed would thus be:

$4(9 - 3p + p^2)$	A <i>Lamarckiana</i>
$4(3p - p^2)$	a "
$3(12 - 3p + p^2)$	A dimorphic trisomic
$3(3p - p^2)$	a "
$(18 - p^2)$	A monomorphic-I trisomic
p^2	a "

The frequency of recessives among the *Lamarckiana* segregates would thus be $\frac{1}{6}(3p - p^2)$, among the dimorphics $\frac{1}{12}(3p - p^2)$, and amongst the monomorphics-I $\frac{1}{18}p^2$. However, when p is small, very large numbers would be required to establish these differences. The total frequency of recessives in all classes would be $\frac{1}{30}(7p - 2p^2)$. The frequencies of recessives among all zygotes and *Lamarckiana* zygotes are given in Table IV.

The frequency of recessives amongst the progeny of trisomics is substantially less than in the progeny of the diploids. But, when p is small, very large numbers would be required to establish differences.

Back-crossing with recessives would seem to be less effective than using selfed populations in the case of trisomics. Thus **Aaa** \times **aa**, in the case of a complex heterozygote, would give equal numbers of **A** and of **a** plants in the progeny. Back-crossing **Aaa** with another structurally homozygous form carrying the recessive factors, e.g. **aa** *blandina*, would give the gametic ratios, but when p was small these would not be greatly different from those in the diploid **Aa**. The whole problem is similar in nature to that of chromatid segregation in structurally normal alethal plants, but of greater difficulty from the numerical standpoint.

CLASSIFIED LIST OF TRISOMICS OF *OENOTHERA LAMARCKIANA*

In the following enumeration only those trisomics of *Oe. Lamarckiana* are recorded which can be placed with some confidence into a particular category. The references to the literature are those embodying data upon which a definite decision could be based. The evidence has chiefly been genetical; occasionally it has been cytological.

Cytological observations upon *Oe. Lamarckiana* trisomics are difficult and precarious, since the maximum configurations possible in any given case are rather rare. It is often quite safe to make deductions when the maximum has not been observed, since a limited series of incomplete configurations specific to each type should be realised. Certain critical

configurations can be relied upon to place different trisomics in their cytological classes.

Thus, the association of thirteen chromosomes and a pair in a dimorphic trisomic may be replaced by a chain of thirteen and a ring pair, a chain of eleven and two ring pairs or a ring of twelve, a univalent and a ring pair. A closed ring of twelve is possible, and not more than two ring pairs are possible.

A monomorphic-V trisomic may show, besides the maximum of a chain of nine chromosomes connecting two ring pairs and a free pair, a chain of thirteen and a ring pair, a chain of eleven and two ring pairs or a chain of nine and three ring pairs. In particular, a closed ring of twelve is impossible, and not more than three ring pairs are possible. It is upon the observation of these two properties by Håkansson (1930) that *Oe. curta* is considered to be a monomorphic trisomic.

A. Dimorphic trisomics:

- (1) Extra chromosome from the *gaudens* complex:

Oe. scintillans de Vries, 1909 *a*. (Extra chromosome carries *n* for *nanella* stature.)

Oe. lata de Vries, 1909 *a*, 1923; Håkansson, 1930.

- (2) Extra chromosome from the *velans* complex:

Oe. cana de Vries, 1916; Håkansson, 1930.

- (3) Nature of extra chromosome unknown.

Oe. pallescens de Vries, 1916; Håkansson, 1930.

Oe. lactuca de Vries, 1916.

Oe. liquida de Vries, 1916; Håkansson, 1930.

Oe. dependens Håkansson, 1930.

Oe. hamata de Vries, 1924 *a*.

Oe. longepetiolata Håkansson, 1930.

Oe. pulla (= *stricta*) de Vries, 1924 *b*; Håkansson, 1930.

Oe. spathulata de Vries, 1924 *a*.

B. Monomorphic-I trisomics:

- (1) *Velans. velans* together with one *gaudens* chromosome; pollen all *velans*:

Oe. oblonga de Vries, 1919; Cleland, 1923 (from *scintillans*).

Oe. albida de Vries, 1923 (from *lata*).

- (2) *Gaudens. gaudens* together with one *velans* chromosome:

Oe. cana nanella de Vries, 1916.

C. Monomorphic-V trisomic:

Oe. curta Håkansson, 1930.

D. Monomorphic trisomics of unknown group; cytology unknown except that they have fifteen chromosomes:

(1) Pollen all *velans*:

Oe. candicans de Vries, 1923.

Oe. auricula de Vries, 1923.

Oe. aurita de Vries, 1923.

Oe. tardescens de Vries, 1924 b.

(2) Pollen all *gaudens*:

Oe. flava de Vries, 1923.

Oe. delata de Vries, 1923.

Oe. nitens (de Vries, 1923) is excluded because it segregates *distans* (de Vries, 1923). The latter has a ring of eight chromosomes and three pairs (Håkansson, 1930). *Oe. nitens* is evidently a half-mutant (*distans*) with an extra chromosome. The behaviour of its chromosomes at meiosis is in harmony with this view (Håkansson, 1930).

SUMMARY

1. The probable nature and breeding behaviour of various trisomics from *Oenothera Lamarckiana* have been deduced from the complex heterozygote and segmental interchange hypotheses.

2. In *Oe. Lamarckiana*, with a ring of twelve chromosomes and a free pair, there are three cytologically distinguishable types of non-disjunction (see Figs. 1-3) which could yield viable eight-chromosome gametes. The non-disjunctions might occur at any one of twelve positions around the ring, so that there would be thirty-six different eight-chromosome gametes. Each could be combined with either *velans* or *gaudens* to give seventy-two zygotic types. There would be a seventy-third type when the plant was trisomic for the free pair chromosome (Fig. 6).

3. The seventy-two trisomics, in which the ring of twelve was disturbed, or such of them as were viable, would belong to six cytologically distinguishable classes (Figs. 4, 5, 7-16). The members of five of these classes (monomorphic trisomics-I-V) would breed true on selfing; one class (dimorphic trisomics of de Vries) would segregate *Oe. Lamarckiana* on selfing.

4. The dimorphics would also segregate a monomorphic-I trisomic on selfing, provided the latter was viable. The monomorphic-I trisomic

pollinated by *Oe. Lamarckiana* should yield *Oe. Lamarckiana*, the monomorphic trisomic and the dimorphic trisomic from which it was derived.

5. The genetic constitutions and breeding behaviour of the different trisomics and the question of trisomic ratios are discussed.

6. The genetic and cytological data are considered in the light of these deductions, especially in relation to the cases of *Oe. scintillans* and *Oe. oblonga*, of *Oe. lata* and *Oe. albida* and of *Oe. cana* and *Oe. cana nanella*.

ADDENDUM

Sterling Emerson (*Amer. Nat.* 69, 545-59, 1935) has recently shown that there are, with respect to aberrations of the ring chromosomes, thirty-six different eight-chromosome complexes possible in *Oenothera Lamarckiana*. Assuming a specific zygotic lethal in each of *velans* and *gaudens*, he shows that there should be forty-two viable trisomics; including the one possible from the pair, there would be forty-three. This interpretation would admit only two monomorphic-I trisomics, one being *velans.velans* with the *gaudens* chromosome carrying the normal allelomorph to the *velans* zygotic lethal, the other being *gaudens.gaudens* with the *velans* chromosome carrying the normal allelomorph to the *gaudens* zygotic lethal. If, as seems likely, my interpretation of *oblonga* and *albida* is correct, it would appear to exclude the possibility of a specific lethal in the *velans* complex, since it is untenable to suppose that two different *gaudens* chromosomes could both include the same normal allelomorph to the *velans* zygotic lethal.

Emerson also supposes that "secondary" trisomic types in *Oenothera Lamarckiana* results from non-disjunctional arrangements in "primary" trisomics. But primary trisomics would not yield through non-disjunction any trisomic types that could not also arise by the same means from normal diploid *O. Lamarckiana*. Admittedly, while the range of trisomics produced by a dimorphic trisomic would be identical with that of diploid *O. Lamarckiana*, the frequencies of different forms should differ. The range would be abbreviated in the case of monomorphic trisomics, any particular monomorphic being incapable of producing, through non-disjunction, anything but monomorphics of a lower order, that is with a shorter chain of chromosomes connecting the two ring pairs.

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THE FREQUENCY OF HETEROZYGOSIS IN FREE-LIVING POPULATIONS OF *DROSOPHILA MELANOGASTER* AND *DROSOPHILA SUBOBSCURA*

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THE evolutionary significance of genetic studies, considered by Bateson (1906) to be one of the main tasks of the then newly founded science, has not been very fully dealt with by the largest school of genetics, that concerned with *Drosophila*. This school has amassed a large volume of important evidence, but the integration of genetics with evolutionary theory has been the work of Fisher (1930, 1931 *a*, 1931 *b*), Haldane (1930, 1932 *a*, 1932 *b*, 1933) and Sewall Wright (1929, 1931, 1934). Tschetverikoff (1926) and Dubinin (1931) have dealt with somewhat more limited aspects.

Following on the theoretical considerations of Tschetverikoff, Timoféeff-Ressovsky (H. A. and N. W. 1927) and Tschetverikoff (1928) inbred populations of *D. melanogaster* to investigate the incidence of recessive mutants. Dubinin and co-workers (1934) have also repeated this on a larger scale, but using somewhat different methods of inbreeding. In this communication further work on the same lines will be described.

MATERIALS AND METHOD

The species inbred were *D. melanogaster* and *D. subobscura* Collin (see note, p. 60). The animals were caught by putting out a milk bottle with a perforated gauze cap, and containing yeasted banana agar. The traps were laid in the grounds of the Biological Field Station of the Imperial College of Science and Technology at Slough in 1933 and 1934. Trapping was commenced at the beginning of June, when only *D. subobscura* was trapped. *D. melanogaster* appeared at the beginning of July, and by the middle of August was the more numerous. In October, however, only *D. subobscura* was trapped. I found that the temperature optimum of *D. subobscura* was much lower than that of *D. melanogaster*. Christie (unpublished) later confirmed this, finding that sterility commenced at about 20° C. This is consistent with the findings of the distribution of the species with season, and the fact that the geographical distribution of *D. melanogaster* is fairly restricted. *D. melanogaster* is

probably killed off during the winter, being reintroduced from food-storage places each year. Some of the *D. subobscura* animals analysed for sex-linked recessives in 1934 were trapped at Dartington Hall, Totnes, South Devon.

Nachtsheim (1928) and Dubinin (1928), extending the work of Nonidez (1920), have shown that the last fertilisation in *Drosophila* replaces earlier ones. A fertilised female will thus produce the offspring of a single male. The possible types derived from a single, wild, fertilised female are shown in Table I.

TABLE I

Possible types derived from single female fertilised in the wild

Wild parents	F_1	F_2 cultures
$AA \text{ } \varnothing \times Aa \text{ } \sigma$	$1 AA : 1 Aa$	25 % all AA , 50 % ($1 AA : 1 Aa$),
$Aa \text{ } \varnothing \times Aa \text{ } \sigma$		25 % ($1 AA : 2 Aa : 1 aa$)
$Aa \text{ } \varnothing \times Aa \text{ } \sigma$	$1 AA : 2 Aa : 1 aa$	Not required
$SS \text{ } \varnothing \times sy \text{ } \sigma$	$Ss \text{ } \varnothing, Sy \text{ } \sigma$	$1 SS \text{ } \varnothing : 1 Ss \text{ } \varnothing, 1 Sy \text{ } \sigma : 1 sy \text{ } \sigma$
$Ss \text{ } \varnothing \times Sy \text{ } \sigma$	$1 SS \text{ } \varnothing : 1 Ss \text{ } \varnothing, 1 Sy \text{ } \sigma : 1 sy \text{ } \sigma$	Not required

A and **S** denote normal autosomal and sex-linked genes respectively, **a** and **s** their recessive allelomorphs. Other types of mating involving recessive genes are much rarer and did not occur in my material. Dominants would be revealed in the free-living animals or in the F_1 .

Realisation of mutants. As will be seen from the data obtained in the investigation, the theoretical 3 : 1 segregation in segregating F_2 cultures is by no means universal, but this does not invalidate the particulate hypothesis utilised in Table I. There are four main factors involved in decreasing the number of mutants in such a segregating culture. These are lower viability of the mutant type, incomplete manifestation (Penetrantz), incomplete expression (Expressivität) (Timoféeff-Ressovsky, 1935), and the association of the mutant gene with a linked lethal carried by the same free-living animal that carries the mutant. These factors may be interrelated, and are also related to culture conditions, viz. temperature, humidity, crowding, etc.

Timoféeff-Ressovsky estimates p (Penetrantz) the manifestation, and e (Expressivität) the expression, as follows. Consider a genotypically homozygous mutant stock of incomplete realisation. Let the numbers of the phenotypes showing the mutant markedly, slightly or not at all be a , b , and c , respectively, then the value of p is given by $\frac{a+b}{a+b+c}$, and the value of e is given by $\frac{a}{a+b}$.

The effect of a linked lethal is rather complex, but its occurrence is somewhat infrequent compared to the other effects, and must be taken

into account. Table II shows the effect of a linked lethal in some of the more probable cases. The mutant genes are denoted by **a** and **l**, and their wild-type allelomorphs by **A** and **L**, **a** and **l** referring to visible and lethal autosomal mutants respectively. If the ratio of lethals to visibles is d , and the frequency of heterozygosis in a free-living population for all visible mutants is F , then the frequency of heterozygosis for lethals is dF .

If the number of pairs of chromosomes is n , the probability that an animal heterozygous for a visible mutant will also be heterozygous for a linked lethal is dF/n . The probability that it will be on the same chromosome as the visible is $dF/2n$, and the probability that it will be on the homologous chromosome is $dF/2n$. The probability that the mate of an animal is heterozygous for a linked lethal is dF/n . The latter case, as we can see from Table II, may result in an expectation of $1/3$ in a segregating F_2 culture. In calculating data for Table II the genetic distance between the visible and the lethal genes is $100c$.

TABLE II

Effect of linked lethal associated with autosomal mutant already detected

	Mating ♀ ♂	Probability of association	Proportion of segregating F_2 cultures	Proportion of visible mutants (genetic distance between a and l is $100c$)
(a)	$\frac{\mathbf{AL\ al}}{\mathbf{AL\ AL}}$	$\frac{dF}{4n}$	$\frac{1}{4}$	$c/3$
(b)	$\frac{\mathbf{AL\ aL}}{\mathbf{AL\ AL}}$	$\frac{dF}{4n}$	$\frac{1}{4}$	$\frac{1}{4}$
(c)	$\frac{\mathbf{al\ AL}}{\mathbf{AL\ AL}}$	$\frac{dF}{4n}$	$\frac{1}{4} \begin{cases} \frac{(1-c)^2}{2} \\ \frac{2c-c^2}{4} \end{cases}$	$c/3$ $\frac{1}{4}$
(d)	$\frac{\mathbf{Al\ AL}}{\mathbf{aL\ AL}}$	$\frac{dF}{4n}$	$\frac{1}{4} \begin{cases} \frac{c^2}{4} \\ \frac{1-c^2}{4} \end{cases}$	$c/3$ $\frac{1}{4}$
(e)	$\frac{\mathbf{AL\ AL}}{\mathbf{aL\ Al}}$	$\frac{dF}{4n}$	$\frac{1}{4}$	$\frac{1}{8}$
	$\frac{\mathbf{AL\ AL}}{\mathbf{Al\ aL}}$	$\frac{dF}{4n}$	$\frac{1}{4}$	$\frac{1}{8}$

The probabilities of the P_1 generation given in Table II are calculated on the basis that one of the parents has already been shown to be heterozygous for a visible mutant, but that the sex of the carrier is unknown.

Dubinín and co-workers (1934) find that for *D. melanogaster* d is 0.4. For *D. subobscura* n is 4, and F (see section on "Estimation of frequency of heterozygosis") is 0.6. The probability that a visible mutant will be associated with a linked lethal is thus 0.06. Similarly in *D. melanogaster* n is 2, and F is 0.9, whence the probability of association with a linked lethal is 0.18. Both these estimates will of course be subject to large sampling errors (see section on "Estimation of frequency").

Table II shows that not only is the expectation in segregating F_2 cultures affected, but the distribution of F_2 cultures for these segregations is also affected.

Let the interaction of all factors, viability, manifestation, expression, association with linked lethals, and environment, decrease the expectation of mutants in a segregating culture from $\frac{1}{2}$ to $\frac{1}{2}r$. We shall call r the *coefficient of realisation*. The main tendency will be for r to be less than 1, but in case (*e*); Table II, r can sometimes be $\frac{4}{3}$ if unaffected by the other factors making up realisation. If realisation is decreased by the effect of a lethal then the maximum realisation is $4c/3$. The effect of a linked lethal cannot be to decrease the expectation slightly in some cultures and greatly in others. Thus bimodal distributions of r will not depend upon the effect of a linked lethal. They will more probably be accounted for by incomplete dominance of the wild-type allelomorph of the mutant, especially since, as will be shown, in most such cases the total number of segregating F_2 cultures is far in excess of the expectations shown in Table II, where this remains unaltered, namely $\frac{1}{2}$.

The nature of the effect upon realisation can be distinguished by the rate of selection to a higher manifestation. If due to manifestation and expression which probably depend upon the genotypic milieu (Timoféeff-Ressovsky, 1935), the rate of selection to a high realisation will be extremely slow compared to the rate of selection if low realisation were due to a linked lethal only, when full manifestation will be attained after one generation of selection.

The explanation of realisation in particular instances is thus a most complex problem and can only be answered by extensive and controlled investigation. In the present investigation such controls were not undertaken, and discussions must be regarded as an attempt to arrive at a first approximation.

The value of r depends very largely upon the subjective elements in the detection of mutants, and may vary considerably from investigator to investigator. The values may, however, be used by the same investigator in comparing parallel cultures.

EXPERIMENTAL PROCEDURE

In 1933 the number of F_2 cultures and the number of flies per culture examined were not fixed. The same applies to the number of males in each F_1 culture. In 1934 I intended examining 100 males in each F_1 culture. In the event of the detection of any mutant form, the females would then be examined so as to distinguish sex-linked from autosomal mutants. For values of r of 0.07, the probability of a sex-linked recessive escaping detection in such a culture, the female parent of which was heterozygous, is 0.02. (For method see section on "Estimation of frequency".) In *D. subobscura* I intended examining 200 animals in each of eight F_2 cultures obtained from paired matings in the F_1 . This would be equivalent to the 100 males in the F_1 . Owing to the unsatisfactory culture conditions this was not always realised, but there were never less than seven F_2 cultures, and the numbers per culture were generally large enough for the detection of mutants of fairly low coefficients of realisation. Those F_2 cultures which yielded less than twenty-five flies were subcultured by mass matings to the F_3 and there examined. In the F_2 of wild *D. melanogaster* females I examined as many flies as possible, usually more than 200 in each of four F_2 cultures derived by random mating within the F_1 . This is equivalent to paired matings in the F_1 , since only the last fertilisation is effective.

The cultures were not kept at constant temperature, since this might bias the result in favour of mutants most pronounced at that particular temperature. *D. subobscura* cultures were at about 22° C. for a week and then at room temperature. *D. melanogaster* cultures were kept at 25–27° C. for 4 days and then transferred to room temperature.

RESULTS OF INBREEDING

(a) Derivation of autosomal recessives

(1) *D. subobscura*, 1933.

Results are given in Table III.

garnet. Eye colour similar to carnation in *D. melanogaster*. 100 per cent. manifestation. Transparent. Reflection points absent. Orange-coloured pigmentation of testes reduced. Difficult to subculture, probably associated with partial sterility of males and short expectation of life. Linked with pointed (see Appendix).

pointed wings. Predominant form pointed wings. More extreme forms dumpy-like and unexpanded. 100 per cent. manifestation. By selection

stocks constant for pointed or for dumpy have been obtained. Different forms probably due to same main gene and different modifiers. Linked with garnet (see Appendix).

TABLE III

D. subobscura, 1933

Mutant	No. of wild females	No. of F_2 cultures without mutants	Average no. of flies in non-segregating F_2 cultures	No. of segregating F_2 cultures	Total flies in segregating F_2 cultures		Coeff. of realisation r
					+	Mutant	
None	8	9.1 \pm 2.0	50 \pm 12	—	—	—	—
garnet eye	1	12	42 \pm 8	2	90	27	0.92
pointed wings	1	10	54 \pm 10	2* {2	128	43	1.0
yellow thorax				{1	41	14	1.0
short-vein _a	1	10	57 \pm 8	3† {3	147	13	0.32
bubble wing				{1	53	9	0.58
crinkly wing	1	4	45 \pm 11	2	97	15	0.53

* Pointed wing and yellow thorax segregated together in one of these cultures.

† Short-vein_a and bubble wing segregated together in one of these cultures.

In other segregating F_2 cultures only one mutant segregated.

yellow thorax. Noticeable in young animals before development of dark pigment which obscures the effect. Wings also yellowish. Reappeared in F_3 culture obtained by mating wild-type sibs of yellow-thorax animals. Manifestation unknown, since attempts at culturing were unsuccessful. Exhibited at meeting of Genetical Society, February 17, 1934.

short-vein_a. Fourth longitudinal vein shortened. Highly variable, sometimes bilateral and sometimes unilateral. After selection realisation improved, p increasing from about 30 per cent. to about 70 per cent. Variability appeared to be due to modifying genes, since homozygous short-vein animals phenotypically short-vein yielded more short-vein animals than homozygous short-vein animals phenotypically wild-type (see Table IV). ($\chi^2=18.5$ $n=1$, $P<0.01$, thus the difference is significant.) The manifestation of females was higher than that of the males in both cases.

TABLE IV

Mating of homozygous short-vein animals

Phenotypes of animals mated	Offspring						\bar{p}
	Females			Males			
	sv _a	+	p	sv _a	+	p	
	sv _a × sv _a	464	62	0.88	312	292	
+ × +	42	24	0.66	32	41	0.44	0.52

In the F_2 of short-vein_a \times pointed and short-vein_a \times scarlet very few short-vein animals appeared (see Appendix). This may have been due

to the presence of wild-type allelomorphs of short-vein_a in the cultures, since short-vein_a is incompletely recessive, and the matings were set up when selection had been in progress only a short while. (Matings of short-vein_a by other mutants occasionally yielded short-vein_a in F_1 .) None the less the figures indicate that gene is linked neither to scarlet nor to pointed. The mutant is probably identical with that found in 1934 (see short-vein_a, 1934).

bubble. Bubble in wing. Reappeared in F_3 cultures obtained by mating wild-type sibs of bubble animals. Manifestation unknown, since attempts at culturing were unsuccessful. Exhibited at meeting of Genetical Society, February 17, 1934.

crinkly. Crinkling of wings. Some animals similar to vesiculated in *D. melanogaster*. Reappeared in F_3 culture obtained by mating wild-type sibs of crinkly. Manifestation unknown since attempts at culturing were unsuccessful. Exhibited at meeting of Genetical Society, February 17, 1934.

(2) *D. melanogaster*, 1933.

Results are given in Table V.

TABLE V
D. melanogaster, 1933

Mutant	No. of wild females	No. of F_2 cultures without mutants	Average no. of flies in non-segregating F_2 cultures	No. of segregating F_2 cultures	Total flies in segregating F_2 cultures		Coeff. of realisation r
					+	Mutant	
None	10	4.5 ± 2.0	102 ± 8	—	—	—	—
rotated abdomen	1	5	118 ± 20	3	154	11	0.27
cross-veinless _d	1	4	169 ± 21	2	351	11	0.12
unexpanded	1	1	117	1	98	4	0.16

cinnabar₂ and ebony appeared in the F_3 .

rotated abdomen. Abdomen rotated, anus to right of middle line. Extremely variable, overlapping with wild-type. Found to be at the same locus as rotated abdomen ($3, 37 \pm$) (see Appendix). May be associated with modifying genes, the absence of which renders it lethal, and the presence of which in large numbers modifies it back to wild-type (Gordon, in manuscript).

cross-veinless_d. Posterior cross-vein absent. Variable. Manifestation greater at lower temperature, both for matings of phenotypically wild-type parents and for matings of phenotypically cross-veinless parents (see Table VI). Manifestation of offspring from phenotypically wild-type

matings less than those from phenotypically cross-veinless matings. Localisation experiments (see Appendix) show gene to be situated at $3, 60 \pm$. Gene not hitherto recorded.

TABLE VI

Mating of homozygous cross-veinless₄ animals

Phenotypes of animals mated	Phenotypes of offspring					
	Warm (26° C.)			Cold (about 18° C.)		
	cv ₄	+	p	cv ₄	+	p
cv ₄ × cv ₄	81	620	0.12	417	34	0.92
+ × +	8	588	0.02	80	287	0.28

unexpanded. Wings fail to expand after emergence. Abdomen more rounded than normal. Resembles pupal (2, $51 \pm$) and is situated close to it (2, $54 \pm$). Matings of pupal and unexpanded yield wild-type. The genes are therefore not allelomorphs. Manifestation in cultures 100 per cent. Low realisation when first derived probably due to association of gene with linked lethal, since selection to 100 per cent. manifestation was immediate (for linkage figures see Appendix).

Mutants derived in F₃. A great many F₂ females derived from different wild originals were inbred to the F₃ in order to investigate a suspected sex-linked sublethal gene for short bristles. The search for the sublethal mutant was not successful, but two mutants were revealed in the F₃.

cinnabar₂ (more intense allelomorph of *cn* (2, 57.5)). Since the number of F₂ cultures examined was six, the probability that the mutant escaped detection in the F₂ owing to chance failure of two heterozygotes mating in the F₁ is $(\frac{3}{4})^6 = 0.15$, which is not low enough to exclude the possibility that the original wild female or her mate was heterozygous for the gene. Localisation experiments (see Appendix) showed that the eye-colour mutant found in this way was a more intense allelomorph of *cinnabar* (2, 57.5). Mutant has 100 per cent. realisation.

ebony. In another F₃ series a dark body colour mutant appeared. In the F₂ of which it was a derivative there were only four cultures. The probability that it escaped notice owing to chance failure of heterozygotes mating in the F₁ is $(\frac{3}{4})^4 = 0.32$. The mutant on being crossed with *ebony* yielded *ebony*, and it was thus regarded as an allelomorph in the *ebony* series.

(3) *D. subobscura*, 1934.

Results are given in Table VII.

short-vein₄. Fourth longitudinal vein shortened. Identical with that

already described (*D. subobscura*, 1933). It is clear from Tables VII B and VIII A that the cultures fall into two distinct groups, one containing cultures yielding high values of r , and the other low values.

TABLE VII A

D. subobscura, 1934

Mutant	No. of wild females	No. of F_2 cultures without mutants	Average no. of flies in non-segregating F_2 cultures	No. of segregating F_2 cultures	Total flies in segregating F_2 cultures		Coeff. of realisation r
					+	Mutant	
None	5	7.6 \pm 0.6	120 \pm 31	—	—	—	—
short-vein _a	1	Appeared in F_1^*		No other mutant in F_2			
rough eye	1	1	25	7	{ 4 high r 3 low r	404 112 171 3	0.86 0.07
cross-veinless } paper }	1	3	159 \pm 40	5†	{ 2 high r 3 low r 1	169 72 421 10 120 12	1.16 0.09 0.36
waxed	1	5	159 \pm 6	3		368 34	0.34
short-vein _b }	1	4	140 \pm 24	3	{ 1 2	222 14 220 18	0.24 0.30
curled					{ 3	366 75	0.68
venae incompletae _a }	1	1	164	6	{ 3 3	414 88	0.70
rough bubble					{ 2	259 41	0.55
eyeless }	1	3	142 \pm 54	5‡	{ 3 2	382 97 256 59	0.81 0.75
rough					{ 4 high r 1 low r 2	545 139 142 3 337 30	0.81 0.08 0.32
shredded						120 23	0.64
venae incompletae _b }	1	2	167 \pm 10	6§			
vermilion							
rough bubble	1	6	176 \pm 13	1			
short-vein _b }	1	Appeared in F_1^*					
outstretched	1	5	123 \pm 35	3		331 48	0.44
shaven	1	Appeared in F_1^*		Not inbred to F_2			

* See Table VII B.

† cross-veinless and paper segregated together in one of these cultures.

‡ eyeless and rough segregated together in two of these cultures.

§ venae incompletae_b and vermilion segregated together in one of these cultures.

In other segregating F_2 cultures only one mutant segregated.

TABLE VII B

Mutant	No. of wild females	Total flies in segregating F_1 culture		Coeff. of realisation r
		+	Mutant	
short-vein _a	1	189	35	0.62
short-vein _b	1	221	12	0.21
shaven	1	36	11	0.93

Timoféeff-Ressovsky (1934) has shown that where there is incomplete realisation of a gene there is also sometimes incomplete dominance of the wild-type. It is thus probable that the short-vein animals in cultures of low realisation were heterozygotes. For the three cultures of high realisation, one F_1 culture and two F_2 cultures, χ^2 is 11.2, for which P is

less than 0.01. These cultures are thus not homogeneous. The difference may be due to the segregation of modifying genes or to temperature effects. Although temperature was not accurately controlled, it was none the less fairly uniform. There is, however, another consideration that must be examined. Phenotypically short-vein animals may be heterozygous as well as homozygous, and phenotypically wild-type animals, whose offspring show the character markedly, may be hetero-

TABLE VIII A

F₂ from wild female yielding in F₁ wild type 96 ♀♀, 93 ♂♂, sv₂ 15 ♀♀, 20 ♂♂

Phenotypes mated in F ₁		1	2	3	4	5	6	7	Total
		sv ₂ × sv ₂	sv ₂ × +	sv ₂ × +	sv ₂ × +	+ × +	+ × +	+ × +	
F ₂	+ + ♀♀	21	39	36	27	53	156	100	432
	♂♂	10	25	46	22	44	160	84	391
	sv ♀♀	10	1	5	14	2	6	4	42
	♂♂	14	2	2	17	0	1	1	37
r		0.44	0.09	0.16	0.78	0.08	0.12	0.11	

TABLE VIII B

Probable genotypes from Table VIII A

	Genotypes of parents of F ₁ culture	Genotypes of parents of F ₁ culture 1	Genotypes of parents of F ₁ culture 4	χ ²	P
(a)	Aa and Aa	Aa and Aa	Aa and aa	11.2	0.01
(b)	Aa and Aa	Aa and aa	Aa and aa	6.7	0.05
(c)	Aa and Aa	Aa and aa	aa and aa	21.2	0.01
(d)	Aa and Aa	aa and aa	aa and aa	7.5	0.05

TABLE VIII c

Mating of sv₂ 1933 collection with sv₂ 1934 collection

	Females			Males			
	sv	+	p	sv	+	p	p̄
(1)	6	7	0.42	3	12	0.20	0.32
(2)	33	21	0.61	15	41	0.27	0.44
(3)	25	1	0.96	39	1	0.97	0.97
Total	64	29	0.69	57	54	0.56	0.59

zygous or homozygous for the gene, if the gene is of incomplete manifestation and incompletely recessive. Thus the calculation of the coefficient of realisation is more complicated than outlined, since the Mendelian expectation will be affected by these considerations. The differences between these cultures may be due to the incorrect determination of the theoretical expectation. In F₂ culture 1 the value of *r* may be either 0.44 as previously given, on the basis that both parents were homozygous for short-vein₂, or 0.88 if only one parent was homozygous and the other

heterozygous. F_2 culture 4 yields a value of 0.78 for r . This might well be 0.39 if the short-vein parent is a heterozygote. Table VIII B gives the values of r corresponding to the various hypotheses of the constitutions of the parents of the F_1 culture and the two F_2 cultures. Incomplete dominance of the wild-type was shown in short-vein_a matings with other mutants, short-vein animals appearing in the F_1 .

The assumptions involved in (b) and (d) of Table VIII B render the series more consistent than those involved in (a) and (c). (b) and (d) correspond to values of r (0.62, 0.88 and 0.78) and (0.62, 0.44 and 0.39) respectively. That the distributions are due to the association with sex-linked lethals is unlikely. In the first instance this could not account for the bimodal distribution of r , since the number of segregating cultures, in this case 100 per cent., is in excess of the expectation for an associated lethal, and secondly the existence of parallel cultures with high and low realisations is even rarer. Thus the cultures of low realisation can only be accounted for by incomplete dominance. The manifestation is also incomplete. The difficulty of determining the genotypes also affects the derivation of pure cultures, where mass mating is resorted to, and the selection of stocks of higher realisation, since phenotypically short-vein animals may harbour wild-type allelomorphs. The presence of wild-type allelomorphs may be responsible for the extremely low number of short-vein animals obtained in the F_2 of short-vein_a × pointed, and short-vein_a × scarlet.

The character appears to be due to the same main gene as the short-vein found the previous year. The results of mating the two short-vein_a stocks are found in Table VIII c. Paired matings were used. The low manifestation in two of the cultures may be due to the segregation of modifying genes, or to the presence of wild-type allelomorphs, or to both. The figures for the last culture would indicate that the character is due to the same main gene. The difference between the sexes is found to be significant ($\chi^2=6.4$, $P<0.02$). This is the same effect as shown in Table IV, but absent from Table V. It may be due to differences in modifying genes.

rough eye. Roughening of surface of eye. Variable. Sometimes only few of the facets are affected. Values of r fall into two distinct groups. The cultures yielding the higher values of r form a homogeneous series ($\chi^2=9.5$, P just exceeds 0.05). In those cultures for which r is 0.07 it seems probable that the rough-eyed animals are heterozygous for rough, i.e. wild-type is incompletely dominant. The probability of finding seven cultures out of eight carrying the homozygous mutant is 0.0004, which

is extremely low. This would support the hypothesis that the rough-eyed animals in cultures of low realisation are heterozygotes. Since from a cursory examination the expression also appeared to be bimodal with regard to the number of facets affected, it is not unlikely that the heterozygous phenotypically rough-eyed animals could be distinguished from the homozygotes by counting the number of facets affected.

Selection of stocks of high realisation was slow, probably due to the difficulty of eliminating wild-type genes from phenotypically rough animals. The males show the character more markedly than the females. Among 589 animals the values of the manifestation were 0.94 and 0.79, and the expression 0.45 and 0.10 for males and females respectively, dividing the mutants into three types, very rough, rough and wild-type. Eventually a stock of very nearly 100 per cent. manifestation was obtained. The presence of a linked lethal is excluded by the large number of segregating F_2 cultures and the slow rate of selection.

purplish. In some rough-eye cultures the colour of the eyes was in some instances darker than expected, even after allowance had been made for age effects. This character was called "purplish". The evidence for such a character was strengthened by the presence of mosaics of this and the wild-type. Since eye-colours, as Timoféeff-Ressovsky (1930) also found in *D. funebris*, are lighter in crowded than in sparse cultures, the separation of slight eye-colour differences, with the culture conditions existing at that time, was extremely difficult. The character purplish has not been investigated further, nor has it been included in the estimation of frequency.

cross-veinless. Posterior cross-vein affected. Extremely variable. Values of r fall into two distinct groups, the lower of which probably indicates incomplete dominance of the wild-type allelomorph. The two cultures giving the higher values of r do not differ from one another significantly ($P=0.20$), nor does the value of r , 1.16, differ significantly from 1 ($P=0.17$).

Subsequent selection showed that the mutant was of incomplete manifestation, which should decrease the value of the coefficient of realisation. The most likely hypothesis, since the manifestation can reach almost 100 per cent. after selection and immediately after derivation was about 80 per cent., is that the high values are due in the first instance to a sampling effect, and in the second instance to the inclusion as cross-veinless of a certain number of heterozygotes due to incomplete dominance of the wild-type allelomorph. Another fact in support of this is that from the result of a mating of cross-veinless animals a phenotypically

wild-type culture was set up in which there was no sign of cross-veinless and from which cross-veinless was never recovered. This shows that the original cross-veinless animals did in fact have wild-type allelomorphs which would have been selected in the subsequent selection for a stock of low manifestation.

The presence of wild-type allelomorphs would also account for other confusing effects in the selection of stocks for different types of expression. Selection to a stock of 95 per cent. manifestation was finally effected.

paper. Texture of wings tissuey. Edges of wings sometimes curled. Eyes and ocelli very slightly lighter. Realisation poor. After selection culture of about 60 per cent. manifestation obtained. Rate of selection slow excluding linked lethal as cause of low realisation.

Waxed. Thorax "matt"-like in appearance (wild-type "glossy"). Wings more opaque, and sometimes somewhat "roofed", and more seldom outstretched. A stock of about 90 per cent. manifestation has now been selected. The rate of selection was slow, thus excluding a linked lethal as cause of the low realisation.

bristle character. In some of the F_2 cultures of the group from which waxed was derived a bristle character much like shaven appeared. There were indications of it again in the F_3 culture obtained by mating the wild-type sibs of these animals, but no animals could be definitely scored as having this bristle character. As other attempts at obtaining it again were also unsuccessful, it has not been taken into account in the estimation of frequency.

short-vein_b. Second longitudinal vein shortened. Extremely variable. A stock of about 70 per cent. realisation has been selected. Identical with mutant of same description found later, as matings between these yielded the character quite markedly.

curled. Wings curled upward. A stock of about 85 per cent. realisation has been selected. Selection was slow, thus indicating no linked lethal.

venae incompletae_a. Fourth and fifth longitudinal and posterior cross-veins affected. In appearance similar to some of the types found in venae incompletae_b found later, but independent of it as matings of the two mutants showed. In many instances inner edge of wing was affected, altering whole shape of wing. Stocks constant for this type were selected. Selection of all stocks of 100 per cent. manifestation was fairly rapid, but since the original realisation did not differ significantly from 1, this does not indicate presence of a linked lethal.

rough bubble. Surface of eye markedly rough. Wings bubble, roof,

curled, notch, or wild-type. Identical in appearance to mutant derived later and taken to be identical. In original cultures males are more frequent than females, but not significantly so. In course of selection this again appeared to be the case, but final figures have not yet been obtained. Females appear somewhat infertile. Stocks are kept going by crossing males to heterozygous females, which might account for the sex difference in later cultures.

eyeless. Extremely variable, ranging from completely eyeless bilaterally to reduced eye unilaterally. The eye is sometimes stalk-like, and occasionally bristles grow out of the eye. Selection resulted in a stock of 100 per cent. manifestation, but expression was not constant. It has, however, greatly improved. Selection was fairly slow, thus excluding association with a linked lethal.

rough. Reappearance of mutant already described.

shredded. Wings shredded. Slightly variable. Stock of 100 per cent. manifestation derived immediately, but as original realisation did not differ significantly from 1 there is no indication of a linked lethal.

venae incompletae. Mainly fourth longitudinal and posterior cross-vein affected. Sometimes second longitudinal affected. The *venae incompletae* animals in culture for which r is 0.08 are probably heterozygotes, i.e. dominance of wild-type allelomorph is incomplete. The value of χ^2 for the other cultures is 13.25 (P less than 0.05). Variation is thus significant. Table IX shows the distribution of the different forms of

TABLE IX

Distribution of vein effects in venae incompletae

Veins affected	1		2		3		4		Total	
	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>
Fourth longitudinal	0.19	0.03	0.06	—	0.10	0.04	0.85	0.00	0.30	0.03
Second longitudinal	0.19	0.30	0.51	—	0.53	0.37	0.15	0.12	0.35	0.26
Posterior cross-vein	0.62	0.67	0.43	—	0.37	0.59	0.37	0.88	0.35	0.71

a. Distribution on derivation.

b. Distribution after 6 months.

the same mutant at time of derivation and 6 months later. In one culture the distribution was reversed. The cross-veinless form appears to be the most stable. The variation is probably due to the segregation of modifying genes, but I cannot explain satisfactorily the changes in distribution. Possible explanations are the greater viability of the cross-veinless forms, or the greater ease of detecting those forms in setting up

cultures, in the course of which more marked types were always selected. Cultures of 100 per cent. manifestation were very rapidly obtained, but as the original realisation was high this has no significance in the detection of a linked lethal.

vermilion. Bright eye colour. Body colour slightly lighter. Stock of 100 per cent. manifestation very quickly derived. Low realisation in original cultures probably due to difficulty of detection and separation in cultures of mixed age, since colour darkens with age resembling young wild-type animals. Low realisation together with rapidity of selection may have been due to association with a linked lethal. Viability of the mutant is also very satisfactory.

rough bubble. Reappearance of mutant already described.

light eye. In some F_2 cultures of the same group from which the second occurrence of rough bubble was noted a slightly lighter eye colour was noted, and this was confirmed in the F_3 where there was some evidence that it was sex-linked. The character was very slight and may have escaped detection in the F_1 . Further investigations were unsuccessful owing to the necessity of proceeding by mass cultures with its attendant effect of overcrowding and obliteration of slight eye-colour differences. This is the only evidence in *D. subobscura* for sex-linked characters.

short-vein_b. Reappearance of mutant already described. Mutant appeared in the F_1 , where matings of short-vein \times short-vein were made. The results of these were 36+ : 23sv_b, 93+ : 59sv_b, 34+ : 8sv_b. Considering these with the animals derived in the F_1 , and those obtained previously, we find that they do not form a homogeneous series ($\chi^2=11.9$, $P=0.02$). This is on the assumption that short-vein animals were homozygous and wild-type animals yielding short-vein were heterozygous. The values of r for the F_2 cultures are 0.39, 0.39 and 0.19, for the F_1 culture 0.22, and for the culture derived earlier 0.24. No hypothesis concerning the real genotype makes these more consistent, so that the differences must be due to other causes, e.g. segregation of modifying genes, temperature, etc.

outstretched. Wings outstretched. Inviabile. Males lived about 4 days, and females about 6 days. Wild-type sibs of outstretched were mated together. Expectation of outstretched $0.1 \times 0.44 = 0.05$. Actual numbers were wild-type 909, outstretched 27, which is in fair agreement considering the care that must be taken to score animals before they die. Effect is genetic and sublethal.

shaven. Thoracic bristles absent. Stock of 100 per cent. manifestation

selected immediately. Selection improved expression. In the course of linkage work commenced about a year after the discovery of the mutant it was found that it is incompletely dominant. Although the mutant was discovered in the F_1 , it is unlikely that the occurrence in approximately one-quarter of the flies was due to its incomplete dominance over the wild-type, one of the parents only being heterozygous, since a stock of 100 per cent. manifestation was derived immediately. The incomplete dominance found later may be due to the different gene complex into which it was crossed, or to dominance having developed in the course of selection for higher expression.

outstretched. In some of the F_2 cultures set up of shaven a great many outstretched animals appeared. In appearance they were similar to those previously described, but their viability was very much better. These were mated to wild-type and an F_2 from this cross was derived, but there was no trace of outstretched. Since quite large numbers of outstretched were obtained, and in different cultures, it is unlikely that it was merely a phenotypic effect. A possible explanation of this phenomenon is that the animals were due to the same main gene as outstretched previously described, but that in the genotypic *milieu* into which they were crossed the gene was now lethal. In the various *milieux* the gene was respectively sublethal, viable, and lethal.

(4) *D. melanogaster*, 1934.

See Table X.

cross-veinless. Posterior cross-vein affected. Variable. This character was later found to be sex-linked. It will be dealt with more fully in the section dealing with sex-linked mutants.

rough eye. Surface of eye rough. Effect very slight. Difficult to select. Difference between the three cultures significant ($\chi^2=80$, $P \leq 0.01$). This may be due to modifying genes and/or incomplete dominance of the wild-type. Association with linked lethal seems unlikely, since both high and low realisations occur in the F_2 cultures. A culture of almost 100 per cent. manifestation has been selected.

brown body. Wing venation also affected. Immediate establishment of culture of 100 per cent. manifestation.

carnation. Eye colour resembling carnation. Low viability. Stock of 100 per cent. manifestation established immediately. Eye darkens considerably with age. Low realisation in original culture probably due to inviability, and difficulty of scoring due to age effects. The low value of r might be due to the association of gene for *carnation*₄ with a sub-

lethal which was the cause of the inviability. More viable stock has since been selected out.

spread. Wings spread. Associated with notched wings and roughened eye surface. A single female occurred in the F_1 . Both males and females sterile. Paired matings of sibs of spread animals showed no sign of the character, although a large number (twenty) of matings was set up. It has, however, recurred repeatedly in carnation_d cultures and in localisation experiments involving carnation_d. The effect is thus clearly not merely phenotypic. It has been taken into account in the estimation of frequency.

TABLE X

D. melanogaster, 1934

Mutant	No. of wild females	No. of F_2 cultures without mutants	Average no. of flies in non-segregating F_2 cultures	No. of segregating F_2 cultures	Total flies in segregating F_2 cultures		Coeff. of realisation r
					+	Mutant	
None	4	4	202 \pm 14	—	—	—	—
cross-veinless _e	1	1	283	3*	219	18	0.30
rough eye					630	75	0.42
brown					185	62	0.91
carnation _d	1	2	167 \pm 57	2†	165	6	0.14
spread					256	23	0.33
extra-vein	1	3	256 \pm 27	1‡	223	8	0.14
ophthalmopedia					227	4	0.069
rough eye	1	1	233	3§	372	60	0.54
eyeless					212	1	0.019
purpur					187	42	0.73
extra-vein	1	2	241 \pm 36	2	188	4	0.083
purplish					347	61	0.60

* cross-veinless_e, rough eye and brown segregated together in one of these cultures.

† spread and carnation_d segregated together in one of these cultures.

‡ extra-vein and ophthalmopedia segregated together in this culture.

§ rough eye and eyeless segregated together in one of these cultures.

|| extra-vein and purplish segregated together in one of these cultures.

In other segregating F_2 cultures only one mutant segregated.

Ophthalmopedia, vermilion_d (autosomal) and baton appeared in the F_3 , and a rough eye (not identical with the one stated above) in the F_4 .

extra-vein. Longitudinal vein growing from mid-portion of posterior cross-vein. It is difficult to select stock of good manifestation. Identical in appearance to that found later, and when mated to it yielded extra-vein. A stock of about 50 per cent. manifestation has been obtained. Owing to slow rate of selection, presence of linked lethal is unlikely.

ophthalmopedia. Corner of eye invaginated. Appendage growing out of invagination. Extremely variable. The main gene appears to be associated with modifying genes since the more marked animals yield stocks of higher manifestation than the less marked. A bar-eyed type was selected out very early and has remained constant. In the ophthalmo-

pedia type selection to begin with increased realisation, but later had the opposite effect. This may be due to more extreme types being sublethal (Gordon, in manuscript).

rough eye. Reappearance of mutant already described.

purpur. Purplish eye colour. Stock of 100 per cent. manifestation selected immediately. Darkens considerably with age. Small deviation of realisation from 1 could be accounted for by difficulty of scoring owing to age effect.

eyeless. Original fly with corner of eye slightly invaginated. Subsequently eyeless animals appeared in offspring. Extremely variable, ranging from normal to eyeless bilateral, with reduced eyes as intermediate types. Subsequently a culture of 80 per cent. manifestation was selected, but this soon reverted back to cultures of low manifestation unless selection was carried on in every generation. The slowness of selection makes a linked lethal unlikely. Identical in appearance to eyeless on fourth chromosome, with which it is probably identical or allelomorphic as preliminary tests show.

extra-vein. Reappearance of mutant already described.

purplish. Slight eye-colour difference. Differs slightly from purpur in appearance, and matings with purpur yielded wild-type. Darkens considerably with age. Stock of 100 per cent. realisation was selected immediately. Low realisation in original culture probably due to difficulty of scoring flies of mixed age groups.

Mutants derived in F_3 cultures. If the number of F_2 cultures per wild female is four, the probability of a mutant for which the female or her mate was heterozygous being missed in the F_3 is $(\frac{3}{4})^4 = 0.32$. This is the probability that a mutant detected in the F_3 had been present as a heterozygote in the original female or her mate. The following mutants have been detected in the F_3 from cultures of the mutants already described.

ophthalmopedia. Segregated from the cross-veinless₄ stock. Reappearance of mutant already described.

vermilion₄. Segregated from the first extra-vein stock. Autosomal eye colour mutant similar to vermilion stock. Effect very slight.

baton. Segregated from first rough-eye stock. Elongated body. Genitalia pushed backward. Anal aperture closed, hence females are sterile. Baton males were crossed to **S Cy/D Sb** females and in the F_1 **Cy Sb** animals were mated together. Here the gene for baton acted as a second chromosome sublethal. The F_2 was **Cy Sb** 196, **Cy** 131, **Sb** baton 6, baton 1. Many extremely elongated dead pupae were noticed. Baton is

probably lethal in the new genotypic *milieu* (Gordon, in manuscript). Further investigations are now proceeding.

*rough eye*₈. Segregated from purpur stock. Matings with rough eye previously described yielded wild-type. Character is more marked than in rough eye previously described.

(b) *Derivation of sex-linked recessives*

See Table XI.

TABLE XI

Incidence of sex-linked mutants, 1934

Species	No. of wild females investigated	Average no. of males examined in each F_1 culture
<i>D. melanogaster</i>	34	83 \pm 4
<i>D. subobscura</i> :		
Dartington	32 }	
Slough	43 }	75 \pm 4

Half the male offspring of a female heterozygous for a sex-linked gene will be mutants. A female fertilised by a mutant male (sex-linked recessive) will not show the character in the F_1 , but the F_2 from such a female will give half mutant males and normal females. It is thus possible to determine by which animal, male or female, the mutant was introduced.

In 1933 no sex-linked mutants were found in either *D. melanogaster* or *D. subobscura*, the number of females inbred being respectively thirteen and twelve. In 1934 a large number of F_1 cultures were examined, and no sex-linked mutants were detected (see Table XI). There was a suspected case of a sex-linked, sublethal associated with a bristle character in *D. melanogaster* in 1933, but this was not verified on subsequent examination. In 1934 there were two cases of sex-linked lethals suspected in *D. subobscura*, but F_3 investigations failed to confirm this. A suspected sex-linked light eye colour of very slight effect has already been described, but this was not investigated further owing to culture difficulties.

The character cross-veinless₆ proved to be sex-linked. The flies in the culture in which it occurred were as follows: wild-type 97 ♀♀, 122 ♂♂, cross-veinless₆ 11 ♀♀, 7 ♂♂. It was assumed, from the nature of its non-occurrence in the F_1 , that it was autosomal, more heavily modified in the males than in the females. Mr F. C. Minns on investigating it found it to be sex-linked (see Table XII A). Calculating the coefficients of realisation in the original F_2 on the basis that it is sex-linked and was introduced by the female, these become $r♀=0.41$, $r♂=0.10$. This

difference was confirmed by matings of homozygous cross-veinless females with cross-veinless males (see Table XII B). Thus the only sex-linked mutant to be detected showed the following peculiarities: (a) in spite of the fact that it was introduced by the female, it was first detected in the F_2 ; (b) the manifestation is decidedly greater in the sex where it can be shielded by its wild-type allelomorph. The importance of these peculiarities will be discussed later.

TABLE XII A

Offspring of $\frac{w fw}{+ +} \frac{+}{cv_e}$ females \times $\frac{+}{Y} \frac{+}{+}$ males

$\frac{w_{(1)} fw_{(2)}}{+ +} \frac{+}{cv_e}$

- (0) No crossing-over.
 (1) Crossing-over between w and fw .
 (2) Crossing-over between fw and we .
 (1) (2) Crossing-over between w and fw and between fw and we .

Males					Females All types
(0), (1), (2), (1) (2)	(0)	(1)	(2)	(1) (2)	
non cv_e	$+ + cv_e$	$w + cv_e$	$w fw cv_e$	$+ fw cv_e$	
505	282	110	59	10	1073

Distance between fw and cv_e = 15 genetic units.
 cv_e is situated at 1, 54 approximately.

TABLE XII B

$\frac{cv_e}{cv_e} \text{♀} \times \frac{cv_e}{Y} \text{♂}$

Females			Males		
cv_e	$+$	p	cv_e	$+$	p
614	370	0.62	341	453	0.43

EFFECTIVENESS OF POLYANDRY

Nonidez (1920), Nachtsheim (1928), and Dubinin (1928) have shown experimentally that the last fertilisation is the effective one. We would thus expect fertilised females to act as if fertilised by a single male. The expectation of heterozygotes in the F_1 , if either male or female were heterozygous for an autosomal recessive, would be $\frac{1}{2}$, and in the F_2 the proportion of cultures revealing mutants would be $\frac{1}{4}$. With effective polyandry the expectation of heterozygotes in the F_1 would be less than $\frac{1}{2}$. Since the expectation in the F_2 is the square of the expectation in the F_1 , we would expect the deviation from $\frac{1}{4}$ in the F_2 to be quite considerable. In estimating the frequency of F_2 cultures showing the mutant we are faced with the same problem as in estimating the frequency of homozygotes due to an autosomal recessive, when families are small, since

a certain number of heterozygotes in a wild population are not detected owing to insufficient number of F_2 cultures set up (see section on "Estimation of frequency of heterozygosis"). Haldane (1932 b) proved the following theorem. "If a group of families derived from normal parents, each family containing at least one abnormal, consists of n_s families of size s , the values of s ranging from 1 to c , and contains R recessives in all, then the most likely value of q , the proportion of normals in a family (supposed constant) is the real root other than unity of the equation

$$\frac{R}{1-q} = \sum_{s=1}^c \left(\frac{sn_s}{1-q^s} \right)$$

and the standard error σ of q (and hence of $p=1-q$) is given by the equation

$$\sigma^{-2} = \frac{R}{q(1-q)^2} - \sum_{s=1}^c \frac{s^2 q^{s-2} n_s}{(1-q^s)^2}.$$

This theorem is applicable to the present case where R is the number of segregating F_2 cultures, s is the number of F_2 cultures per wild female inbred, and n_s the number of wild females corresponding to s , p is the proportion of segregating F_2 cultures, and q the proportion of non-segregating F_2 cultures.

Some F_2 cultures show a bimodal distribution of r , the coefficient of realisation, probably due, as stated previously, to the fact that the dominance of the wild-type allelomorph of the recessive gene is incomplete. Cultures of low realisation in such bimodal distributions are re-

TABLE XIII

Distribution of F_2 cultures, 1934

Species	F_2 cultures. No. yielding homozygous mutants.	No. of wild females	Total n_s	p	
	Total				
<i>D. subobscura</i>	1/8	1	9	0.32 ± 0.05	
	2/8	4			
	3/8	2			
	4/8	1			
	5/8	1			
	1/7	1	5		
	2/7	1			
	3/7	3			
	<i>D. melanogaster</i>	1/4	7		10
		2/4	2		
3/4		1			
Both				0.30 ± 0.04	

Figures are derived from Tables III, V, VII A, and X. For explanation see text.

garded as not showing the gene in the homozygous condition, and thus as non-segregating cultures for the homozygous mutant (see Table XIII). For *D. subobscura* the ratio of the deviation from 0.25 to the standard error is 1.4, for *D. melanogaster* 0.3, and for the two together 1.3, none of which are significant. The distribution of segregating F_2 cultures is consistent with the absence of effective polyandry, and since the deviation of the estimate of p is positive, inconsistent with any but extremely small frequencies of multiple fertilisation.

FREQUENCY OF HETEROZYGOSIS IN THE POPULATION

Suppose a female caught, and the male by which she was fertilised, are both heterozygous for a recessive mutant (heterozygote \times heterozygote mating). The expectation of mutants is $r/4$, where r is the coefficient of realisation. If the size of an F_1 culture is t , the probability of finding at least one recessive is $1 - (1 - r/4)^t = 1 - j$ (say).

Suppose the female caught or the male fertilising the female to be heterozygous, and the other partner to be homozygous (heterozygote \times homozygote mating). The mating in the P_1 is $AA \times Aa$. The constitution of F_1 is $1AA : 1Aa$. The mating in F_1 is

$$1AA \times AA : 2AA \times Aa : 1Aa \times Aa,$$

i.e. $\frac{1}{4}F_2$ cultures will be segregating for the mutant. Let n be the number of F_2 cultures, s be the size of each F_2 culture, s_m be the size of the m th F_2 culture, and r be the coefficient of realisation. The probability that there will be no recessive mutant in the m th F_2 cultures is $\frac{3}{4} + \frac{1}{4}(1 - r/4)^{s_m}$. The probability that there will be none in n cultures is

$$\prod_1^n [\frac{3}{4} + \frac{1}{4}(1 - r/4)^{s_m}] = k \text{ (say).}$$

Thus the probability of finding at least one recessive is $1 - k$. Let F be the frequency of heterozygosis for a given mutant in the population, i.e. frequency of mutant genes $f = F/2$. Let p be the frequency of double heterozygote matings, and q be the frequency of heterozygote \times homozygote matings for each mutant. Then $F = p + \frac{1}{2}q$.

$p = \frac{1}{N} \sum_1 \frac{1}{1-j}$, where N is the number of wild females inbred to the F_1 and \sum_1 is the sum over all cases where the mutant shows in the F_1 . (The observed value of p would be $\frac{1}{N}$, and the value given above is the

corrected value allowing for the possibility of mutants being missed owing to the size of the culture.)

$q = \frac{1}{N_B} \sum_2 \frac{1}{1-k}$, where N_B is the number of wild females inbred to the F_2 , and \sum_2 is the sum over all cases where a mutant shows in the F_2 and not in the F_1 . $\left(\sum_2 \frac{1}{1-k}\right)$ is a correction factor corresponding to $\sum_1 \frac{1}{1-j}$.

The estimate of F is $\frac{1}{N} \sum_1 \frac{1}{1-j} + \frac{1}{2N_B} \sum_2 \frac{1}{1-k}$.

Each mutant discovered in the F_2 contributes to the frequency of heterozygosis for that mutant $\frac{1}{2N_B(1-k)}$. The probability of this occurring is $q(1-k) + pj$, where $q(1-k)$ would be the apparent value of the frequency of heterozygote \times homozygote mating, and pj the corresponding value of the frequency of heterozygote \times heterozygote mating which escaped detection in the F_1 .

Each mutant discovered in the F_1 contributes to the frequency of heterozygosis for that mutant $\frac{1}{N(1-j)}$. The probability of this occurring is $p(1-j)$ which is the apparent value of the frequency of heterozygote \times heterozygote mating.

Now there are two types of experiment, type A, in which inbreeding is carried to the F_1 only, and type B in which inbreeding is carried to the F_1 and F_2 .

For type A experiments we have the following:

Contributions to frequency	0	$\frac{1}{N(1-j)}$.
Probability	pj	$p(1-j)$.

From a consideration of the first and second moments we get:

$$\begin{aligned}\text{Mean} &= \frac{p}{N}. \\ \text{Variance} &= \frac{p}{N^2(1-j)} - \frac{p}{N^2}.\end{aligned}$$

For type B experiments we have the following:

Contributions to frequency	0	$\frac{1}{2N_B(1-k)}$	$\frac{1}{N(1-j)}$.
Probability	$1-p-q(1-k)$	$q(1-k) + pj$	$p(1-j)$.

From a consideration of the first and second moments we get, neglecting pj as small compared to $q(1-k)$:

$$\text{Mean} = \frac{q}{2N_B} + \frac{p}{N}.$$

$$\text{Variance} = \frac{q}{4N_B^2(1-k)} + \frac{p}{N^2(1-j)} - \left\{ \frac{q}{2N_B} + \frac{p}{N} \right\}^2.$$

Weighting each of these, where N_B is the number of wild females inbred in type B, N_A is the number of wild females inbred in type A experiments, and N the total number of females inbred is $N_A + N_B$; the mean is

$$\frac{q}{2} + \frac{N_B}{N} p + \frac{N_A}{N} p = \frac{1}{2}q + p.$$

Weighting variance and summing over all cases (over all flies inbred):

$$\text{Variance} = \frac{q}{4N_B^2} \sum_B \frac{1}{1-k} + \frac{p}{N^2} \sum_N \frac{1}{1-j} - \frac{p^2}{N} - \frac{pq}{N} - \frac{q}{4N_B},$$

where \sum_B is summation over cases where inbreeding is carried to the F_2 as well as to the F_1 , and where \sum_N is summation over all cases where inbreeding is carried to the F_1 .

Now the quantity $(1-r/4)^s$ is found to be negligible except where $r < 0.15$. Thus $\sum_1 \frac{1}{1-j} = 1$, since no mutants of low realisation were found in the F_1 , and $\sum_N \frac{1}{1-j} = N$, since the mean value of r was also > 0.15 .

In the *D. melanogaster* 1934 collection some mutants of very low realisation were found, and the effect of r was included in the estimate of k .

$\sum_2 \frac{1}{1-k}$ was calculated directly.

$\sum_B \frac{1}{1-k}$ was calculated for these mutants by taking the value of $\frac{1}{1-k}$ in the particular experiment as the value over all the experiments, since n was constant, namely 4.

$$\text{i.e.} \quad \sum_B \frac{1}{1-k} = \frac{N_B}{1-k}.$$

In all other groups the value of k depends on the number of F_2 cultures for each wild female, i.e. is $(\frac{1}{4})^n$.

Had the total number of flies investigated been large, p and q would

have been calculated directly from the experiment, but since this was not the case, the assumption of random mating was made, whence

$$p = F^2,$$

$$q = 2F(1 - F).$$

The frequencies of the heterozygotes and their sampling variances are given in Table XIV.

TABLE XIV

*Frequency of heterozygotes in populations**

Species	Year	Mutant for which animals are heterozygous	Frequency	Variance	
<i>D. sub-obscura</i>	1933	garnet	0.045050	0.0022973	$N = N_B = 12$. Frequency of all heterozygotes = 0.277 ± 0.117
		pointed	0.043030	0.0021989	
		yellow thorax	0.043030	0.0021989	
		short-vein _a	0.042423	0.0021728	
		bubble	0.042423	0.0025829	
		crinkly	0.050687	0.0025695	
<i>D. melano-gaster</i>	1933	rotated abdomen	0.042741	0.0025558	$N = N_B = 13$. Frequency of all heterozygotes = 0.177 ± 0.100
		cross-veinless _d	0.046790	0.0027860	
		unexpanded	0.087912	0.0050036	
<i>D. sub-obscura</i>	1934	short-vein _a	0.031250	0.0010907	$N_B = 15$, $N = 32$. Frequency of all heterozygotes = 0.636 ± 0.149
		short-vein _b	0.069718	0.0022569	
		shaven	0.031250	0.0010907	
		rough eye	0.078084	0.0023709	
		cross-veinless	0.037042	0.0012749	
		paper	0.037042	0.0012749	
		waxed	0.037042	0.0012749	
		eyeless	0.037042	0.0012749	
		shredded	0.037042	0.0012749	
		venae incompletae _b	0.037042	0.0012749	
		vermilion	0.037042	0.0012749	
		outstretched	0.037042	0.0012749	
		curled	0.038468	0.0013238	
		venae incompletae _a	0.038468	0.0013238	
		rough bubble	0.076936	0.0024559	
<i>D. melano-gaster</i>	1934	rough eye	0.16254	0.0095621	$N_B = 9$, $N = 34$. Frequency of all heterozygotes = 0.924 ± 0.251
		brown	0.08127	0.0056121	
		carnation _d	0.08181	0.0056843	
		spread	0.08127	0.0056121	
		extra-vein	0.16317	0.0096125	
		eyeless	0.10965	0.0098533	
		purpur	0.08127	0.0056121	
		purplish	0.08127	0.0056121	
		ophthalmopedia	0.08194	0.0057003	

* Data on which the calculations are based are in Tables III, V, VII, and X.

COMPARISON OF *D. MELANOGASTER* WITH *D. SUBOBSCURA* (1934)

COLLECTION

$$\frac{\text{Diff. in frequencies}}{\sqrt{\text{Sum of variances}}} = 1 \text{ approx.}$$

The frequencies of heterozygosis do not therefore differ significantly. There are, however, in *D. melanogaster* mutants whose realisation is

sufficiently low to affect the values of k . These were characterised by an association with modifying genes, except for carnation_d, where the low realisation was due to other causes. These mutants are extra-vein, ophthalmopodia and eyeless. The total frequency of these is 0.36 ± 0.16 . This type has not been detected in *D. subobscura*, but as the sampling error of the estimate of frequency in *D. melanogaster* is extremely high, it is not possible at this stage to give a reasonably accurate answer to the question of their existence in *D. subobscura*. The further investigation of this type of mutant is indicated. With regard to wing forms the position is reversed, the frequency of heterozygosis for these in *D. subobscura* being 0.21 ± 0.08 , and zero in *D. melanogaster*, if we except extra-vein which has an exceptional realisation. Here again owing to paucity of numbers no definite conclusions can be drawn. With regard to body and eye-colour mutants, a significant difference in frequencies, which is not the case in this instance, since $\frac{\text{Diff. in frequencies}}{\sqrt{\text{Sum of variances}}} = 1.4$, would not

necessarily indicate a difference with regard to this type of mutant, but might be a consequence of the difficulty of detecting this type of mutant in *D. subobscura*, owing to the obscuring effect of the black pigmentation. Spencer (1935) and Timoféeff-Ressovsky (H. A., 1930) note differences between *D. melanogaster* and *D. funebris*. *D. subobscura* shows some good mutants of the *D. melanogaster* type and poor mutants of the *D. funebris* type, but it is premature to state to which of the two species it bears the closer resemblance.

COMPARISON BETWEEN THE 1933 AND 1934 POPULATIONS OF *D. MELANOGASTER* (BOTH TRAPPED AT SLOUGH)

Since technique had improved between 1933 and 1934, the number of mutants detected would differ for exactly the same sample. The type of mutant detected in 1933 would, however, still be detected in 1934, so that an examination of differences in frequencies for the particular mutants detected in 1933 is valid. The frequency of heterozygosis for the three mutants of the 1933 investigation from which the estimate of heterozygosis was made is 0.18 with variance 0.0103, while the frequency of heterozygosis for these same mutants in 1934 was zero. Consider now a sample from the same 1933 population which yields none of these mutants. The frequency may be considered to be 0 with a variance of 0.0103. The value of $\frac{\text{Diff. in frequencies}}{\sqrt{\text{Sum of variances}}}$ is 1.4, which is not significant.

The exact treatment of the 2×2 table for the condition of 0 heterozygotes in a similar sample under the same conditions gives $p=0.11$, which is also not significant. The 1934 conditions were less suitable for the detection of the type of mutant found in 1933, since less flies were inbred (nine instead of twelve) and many less F_2 cultures were derived per fly. The differences between the 1933 and 1934 cultures are *a fortiori* not significant.

COMPARISON BETWEEN THE 1933 AND 1934 POPULATIONS OF
D. SUBOBSCURA (BOTH COLLECTED AT SLOUGH)

Of the 1933 mutants only short-vein_a reappeared in 1934. As shown above, comparison between the two populations for mutants found in the year of indifferent technique is valid.

$\frac{\text{Diff. in frequencies}}{\sqrt{\text{Sum of variances}}} = 2.00$. This is just significant. Since, however, the variances are relatively high compared to the frequencies, for significance we should take values which are well in the region of significance. Thus the evidence for differences between the populations in successive years is not very decisive.

DISCUSSION

There has been comparatively little work done on the incidence of recessive mutants in wild populations. In a great many genetical investigations it is clear that certain recessive characters were present in the original stock and were revealed by inbreeding, but in very few cases have systematic records been kept with a view to the examination of such variability as can be studied through visible mutants. A search through the genetic literature with this particular object in view would most probably reveal many such cases. A cursory glance through the monographs on *D. melanogaster* reveals that a great many mutants were derived from back-cross experiments which involve inbreeding. Certain mutants, such as white ocelli, were only observed after considerable experience in the detection of mutants had been gained. It is quite conceivable that wild-type stocks previously regarded as homozygous would reveal mutants on inbreeding. The frequency of heterozygosis for such mutants can only be obtained by systematic inbreeding which has seldom been undertaken.

In *Gammarus* such records of inbreeding have been made by Spooner (1932) who finds that approximately five out of twenty-six animals are heterozygous for some recessive gene. Jenkin (1930) has conducted in-

breeding experiments with a view to the production of economically advantageous types. The plants used propagated normally by out-crossing. The plants were selfed artificially, and 67 per cent. of the seedlings, derived from both commercial and indigenous seeds, of *Lolium perenne* were found to be defective. Similar results were obtained from *Lolium perenne multiflorum*, from *Phleum* and from *Festuca rubra*. Sansome and LaCour (unpublished) have inbred *Chorthippus parallelus*, but have found no mutant types beyond those visible in the free-living population which is polymorphic.

In *Drosophila* systematic work was initiated by Tschetverikoff (1928), who commenced inbreeding at the same time as his theoretical work was published. A condensed account only is available (1928) and it does not state the nature of all the mutants derived. Timoféeff-Ressovsky (H. A. and N. W., 1927) also inbred a population from a garbage heap in a Berlin garden. Finally Dubinin and co-workers (1934) inbred samples from a large number of populations over two successive years in order to study what he termed (1931) the "ecogenotypes". Since Dubinin and co-workers used a modified method of inbreeding involving balanced lethals, thus enabling the chromosome to be marked coincidentally with the detection of mutants, their records are of the number of altered chromosomes and not of heterozygotes, as in this investigation. The method has three advantages over my own methods: no correction of the estimate of frequency is necessary, the chromosome on which the mutant is situated is known simultaneously with the detection of the mutant, and lethals on the second chromosome can be detected. A disadvantage is that the method involves outcrossing and hence we do not get a picture of the mutant in its own genetic *milieu*. It is difficult to estimate the relative efficiencies of the two methods for unusual associations with modifying genes of the types indicated in rotated abdomen and ophthalmopodia. If the theory that association with many modifying genes reduces certain mutants to wild-type and extensive dissociation from these produces lethals is correct, the effect of outcrossing would be to render the gene lethal, and if on the second chromosome it would be recorded as such. On the other hand, for the ophthalmopodia type which improves in manifestation when dissociated from modifiers, outcrossing would be advantageous, rendering it more detectable. Dubinin and his co-workers do not give the detailed derivation of each mutant and thus do not discuss the realisation of mutants, with the exception of extra-bristles.

In comparing results, the difference in methods must be taken into

account, as well as the more disturbing subjective factor of differences in skill of detection between different observers. I am fairly skilled in the detection of eye colour and eye surface mutants, but am weak at bristle effects, while presumably the Russian school is skilled at the latter.

Dubinín and co-workers analysed 1900 *X*-chromosomes and 2400 autosomes. The percentage of altered autosomes, corresponding to the percentage of heterozygotes, was found to range between 0 and 30. As the number of wild females investigated was large the sampling variance of this estimate is much smaller than of my estimate. The difference would probably be significant had the methods of detection been comparable, which is certainly not the case. Dubinín and co-workers do not record mutants of low realisation. Among the 1900 sex-chromosomes investigated, no sex-linked mutants visible or lethal were detected. This is confirmed by the present investigation both in *D. melanogaster* and in *D. subobscura*, with the exception of the sex-linked cross-veinless found in *D. melanogaster*, where there appears to be a special mechanism protecting the males against selection. Since the probability of survival of this mutant is greater if the gene is less effective when haploid than when diploid, the gene may be either an antimorph or a neomorph (Muller, 1932).

Dubinín has not found any autosomal dominants, sex-linked dominants, or sex-linked recessives, agreeing with my own findings—with the notable exception dealt with above, and of shaven which was not appreciably dominant at the time of derivation—and disagreeing with the findings of Timoféeff-Ressovsky. In the latter's work, of the forty-eight occurrences of mutants twenty-nine are autosomal dominants, sex-linked dominants, and sex-linked recessives both visible and lethal. It is true that some of his dominants are weak, but none the less his findings are surprising. It is clear from the work of Haldane (1927) that these could not maintain their frequency except at extremely high mutation rates. The conditions in a garbage heap do not seem likely to produce these.

The following is derived from Haldane's treatment.

If the relative fitnesses are

$$AA : Aa : aa :: 1 : 1 - K : 1 - k \text{ and } Ay : ay :: 1 : 1 - k,$$

and mutation rate is M , neglecting back mutation rate as small in comparison, then for autosomal genes the frequency of the gene x is given by the equation

$$(k - K)x^2 + Kx - M(1 - K) = 0,$$

whence if K is much less than k ,

$$x = \frac{1}{\frac{K}{2M} + \left(\frac{k}{M}\right)^{\frac{1}{2}}}.$$

Now if K is very small this becomes $(M/k)^{\frac{1}{2}}$. If, however, there is selection on the heterozygote this becomes $2M/K$, which is much less than $(M/K)^{\frac{1}{2}}$. Thus mutants which are completely recessive will be far more numerous than the incompletely recessive, and *a fortiori* than the real dominants.

The frequency x of sex-linked recessive mutants is given by the equation

$$2kx^2 + kx - 3M = 0,$$

whence $x = 3M/k$, approximately.

Thus we would expect very many more autosomal recessives than autosomal dominants, sex-linked recessives, and *a fortiori* sex-linked dominants, while Timoféeff-Ressovsky finds that the less probable types predominate.

Timoféeff-Ressovsky also finds that of the six occurrences of a sex-linked visible recessive four were introduced by the male since they were detected in the F_2 , and two were introduced by the female and so detected in the F_1 , which is again contrary to the expectation that the great majority of the carriers would be of the sex diploid for the locus. Finally Timoféeff-Ressovsky finds evidence for multiple fertilisation of females, contrary to the findings of Dubinin and Nachtsheim. I find it extremely difficult to reconcile Timoféeff-Ressovsky's findings with those of other workers, including myself.

It is difficult to decide whether the absence of recessive genes in *Chorthippus parallelus* found by Sansome and La Cour is definitely significant, since differences of training in detection of mutants are important. There is, however, a high probability that obvious mutants would have been detected had they been present.

The difference may be due to:

- (1) Differences in stability of chromosomes, mutation rates, etc.
- (2) Differences in mating system operating. *Chorthippus parallelus* may be more inbred.
- (3) Different type of protection against selection. Fisher (1931 b) has suggested that in polymorphic species the heterozygotes are fitter than either of the homozygotes. Such a development of fitness of the heterozygote may be accompanied by the absence of the phenomenon of dominance and its attendant shielding effect against selection.

The distribution of F_2 cultures yielding mutants conforms both to the view that multiple fertilisation of the females is not effective, and to the view that the free-living animals from which they were derived were heterozygous for the recessive gene. The incidence of mutants is much higher than that due to spontaneous mutation after laboratory culture. It may be argued that the period immediately following laboratory culture was the time in which such changes took place, the change rather than the laboratory conditions themselves causing instability. This is, however, untenable since only drastic treatment (irradiation, heat, and cold) produce such changes, and the change from free-living state to laboratory conditions could hardly be compared to these. Also the extent to which they occur is incomparably greater, and is restricted to the autosomal mutants, which is not the case in artificially induced mutations. Although the view that mutants are laboratory artefacts is not generally held any longer, this present investigation should be of some value to those who hold it.

The high incidence of modifying genes, and the indications of incomplete recessivity in free living mutants, indicate a fruitful field of study on the evolutionary significance of these, with the implied bearing on the evolutionary theories of Fisher (1931 *a*, 1931 *b*), Haldane (1930, 1932, 1933) and Sewall Wright (1929, 1931, 1934).

Finally, just as irradiation by X-rays provides the best means of obtaining sex-linked recessives in *Drosophila*, so clearly inbreeding provides the best means of obtaining autosomal recessives.

SUMMARY

1. Free-living populations of *D. melanogaster* and *D. subobscura* show a considerable incidence of heterozygosis for autosomal recessives. In 1933 frequencies were *D. subobscura* 0.277 ± 0.117 , *D. melanogaster* 0.177 ± 0.100 , and in 1934 *D. subobscura* 0.636 ± 0.149 and *D. melanogaster* 0.924 ± 0.251 . Differences between successive years are largely accounted for by improvements in technique.

2. In *D. subobscura* no sex-linked mutants were found, and in *D. melanogaster* only one. No dominants, sex-linked or autosomal, were found in either species; one mutant, shaven, in *D. subobscura* exhibited incomplete dominance only after selection for high expression.

3. The realisation of mutants was noted and a coefficient of realisation of each mutant was calculated. A great many mutants of low realisation were found. In many cases the low realisation was shown to be due to the action of genes which modified mutants back to wild-type.

4. Of the mutants found in 1933 in *D. melanogaster* two are hitherto unrecorded, one is a more intense allelomorph of cinnabar, one an allelomorph in the ebony series, and one, rotated abdomen, at the same locus as the rotated abdomen already known. The investigation of the identity of the 1934 mutants is not yet completed.

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APPENDIX

Localisation of cinnabar₂ (D. melanogaster)

$$\frac{\text{b}_{(1)} \text{pr}_{(2)} +}{+ + \text{cn}_2} \text{♀} \times \frac{\text{b pr cn}_2}{\text{b pr cn}_2} \text{♂}$$

(0)	(0)	(1)	(1)	(2)	(2)	(1) (2)	(1) (2)
b pr +	+ + cn₂	+ pr +	b + cn₂	b pr cn₂	+ + +	b + +	+ pr cn₂
1578	1553	108	71	29	27	3	0

Genetic distance: **pr/cn₂** = 1.5.

Locus of **cn₂**: 54.5 ±. Locus of **cn₁**: 56.

F₁: **cn₁ × cn₂**, intermediate between the two.

F₂: **cn₁ × cn₂**, **cn₁ 97 × cn₂ 31**.

Localisation of unexpanded (D. melanogaster)

$$\frac{+_{(1)} \text{vg}_{(2)} \text{sp}}{\text{u} + +} \text{♀} \times \frac{\text{u vg sp}}{\text{u vg sp}} \text{♂}$$

(0)	(0)	(1)	(1)	(2)	(2)	(1) (2)	(1) (2)
+ vg sp	u + +	u vg sp	+ + +	+ vg +	u + sp	u vg +	+ + sp
193	199	34	33	98	102	10	12

Genetic distance: **u/vg** = 13 units.

Locus of **u**: 54 ±. Locus of **pu**: 51 ±.

Mating **u × pu** yielded wild-type only.

Localisation of cross-veinless_d (D. melanogaster)

P₁: **cv_d × S Cy/D**.

F₁: **Cy D × Cy D**.

Cy D	CyD cv_d	Cy cv_d	Cy	D	D cv_d	cv_d	wt
68	2	43	12	58	0	24	10

Distance from **D** approx. 20 units.

$$\frac{\text{ru cv}_d}{+ +} \text{♀} \times \frac{\text{ru cv}_d}{\text{ru cv}_d} \text{♂}$$

ru cv_d	+ +	+ cv_d	ru +
329	462	289	302

Since there is an overlap with wild-type only figures for cross-veinless were used.

Genetic distance **ru/cv_d** = 47 units. Since the value given is so large the distance may be anything from 40 upwards.

The linkage figures with Dichaete, however, would indicate that it

was to the right of *Dichaete* since 20 units to left of *Dichaete* would bring it to the neighbourhood of 25. Thus locus of cross-veinless is approx. 60.

The gene is independent of *cv*₆ at 58.3.

Localisation of rotated abdomen (D. melanogaster)

	$\frac{\text{ru rot}}{+ +} \text{♀} \times \frac{\text{ru rot}}{\text{ru rot}} \text{♂}$		
ru rot	+ +	ru +	+ rot
256	449	235	148

Genetic distance between **ru** and **rot** $35 \pm$.

Mating with rotated abdomen at $37 \pm$ showed offspring rotated.

Thus mutants are at the same locus.

Ebony. Dark animals identical in appearance with ebony mated to ebony yielded ebony.

Linkage of short-vein_a with scarlet and pointed (D. subobscura)

(Scarlet obtained from A. H. Sturtevant)

*F*₂: short-vein \times scarlet (**st** \times **sc**) gave

wt	sc	st	sc st
605	184	41	3

*F*₂: short-vein \times pointed (**st** \times **p**) gave

wt	p	st	st p
622	189	24	1

Short-vein appears to be linked to neither pointed nor scarlet, but the figures are not decisive for linkage with pointed.

Linkage of scarlet, pointed and garnet (D. subobscura)

*F*₂: **sc p** \times **g**.

wt	sc	p	g	sc p	sc g	p g	sc p g
202	60	67	62	20	16	0	0

Pointed is clearly linked to garnet.

$\frac{\text{g p}}{+ +} \text{♀} \times \frac{\text{g p}}{\text{g p}} \text{♂}$	
g p 48, + + 59, g + 33, + p 37	

Recombination value of garnet and pointed = 39 per cent.

NOTE. *Drosophila subobscura* sp.n. ♂ ♀. By J. E. Collin, F.R.E.S. A common and widely distributed species hitherto included under *D. obscura* Flin. which it generally resembles, though easily distinguished in the male sex by its larger tarsal "combs" on first and second joints of front tarsi (that on first joint occupying about apical half, on second joint more than apical half), while in both sexes the thorax is uniformly light brownish grey without even faint indications of darker stripes. Length about 2 mm.

A REPETITION OF McDOUGALL'S LAMARCKIAN EXPERIMENT

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(With Eleven Text-figures and Five Pedigree Charts)

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I. INTRODUCTION

PROFESSOR WILLIAM McDOUGALL has been conducting, since 1920, an experiment devised to examine the validity of the Lamarckian hypothesis, and has obtained results, as revealed in the three reports that have appeared (McDougall, 1927, 1930; Rhine and McDougall, 1933), that are impressive and seem to warrant the interpretation presented. It is difficult for the critic to find flaws in the techniques that McDougall has so patiently developed, and it is impossible not to be impressed by the scientific quality of the reports themselves.

Reading the earlier reports, I found it impossible to overthrow McDougall's conclusions by argument. Yet, though I could not deny that McDougall was possibly justified in so regarding them, I could not bring myself to accept the results he had obtained as satisfying evidence of the reality of Lamarckian transmission. I formed the opinion that his conclusions would be shown, by further experimentation, to be unwarranted, for I had become more and more critical of McDougall's use of controls, of his neglect to maintain pedigrees and individual records,

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and of his method of presenting his data. This being so, there was nothing left for me to do save to repeat the experiment myself.

McDougall trained rats by a standardised procedure to avoid the brightly lit and electrified route out of a water tank. The rat is lowered by hand gently into the middle blind compartment of the tank out of which there are two lateral ways leading to platforms, one of these, alternately on the right and left, being illuminated and so wired that the rat, stepping on to it out of the water, receives an electric shock. The tank is such that the rat has the choice of leaving the water either by way of the dim platform and thence out of the tank without receiving a shock, or else by way of the lit platform and getting a shock.

In order to accustom the rat to the conditions of the experiment, each is given 6 runs in the tank with the light alternating but without shock as a preliminary to the actual training period, during which each rat is placed in the tank 6 times daily. On the first of these occasions the light and shock are on the right of the rat as it swims down the middle blind compartment, on the second they are on the left, on the next they are on the right, and so on, and this practice is continued until the rat has mastered the task; that is, until it has left the tank 12 times in succession by the dim, unshocked route. The training is completed when the rat "learns to discriminate between the bright and the dim gangway (in anthropomorphic terms it learns to accept the bright light as a signal warning it of a shock)".

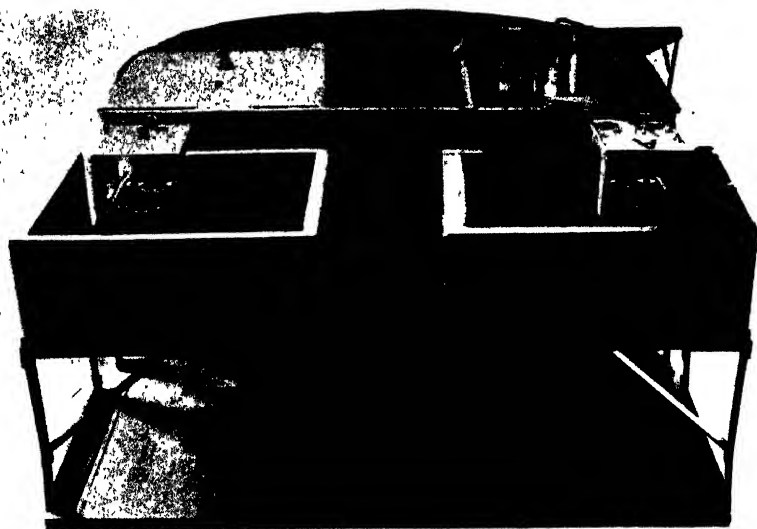
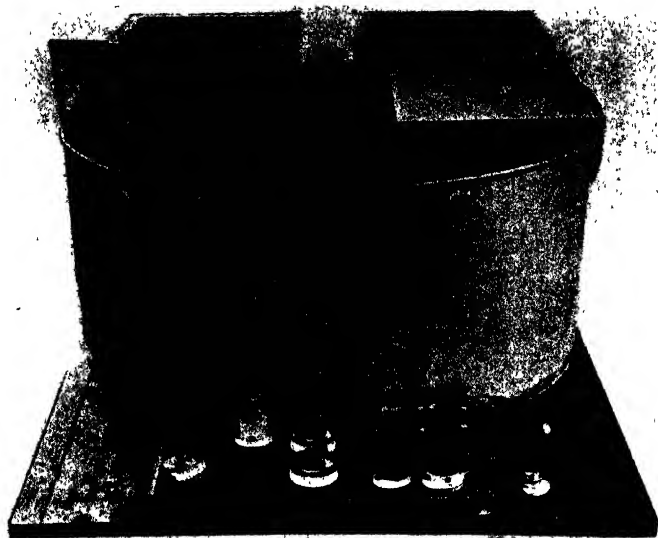
Up to the time of the third report 34 successive generations of rats of the tank-trained stock, and a number of controls (rats whose ancestors were not so trained) have been trained, and the facility in learning has been measured by the number of errors made (shocks received) by the rat before it learns to avoid the light and shock and always to choose the dim, unshocked exit. The main conclusions to which McDougall has arrived are: (1) with the passing of the generations the average number of errors per rat made by individuals of the tank-trained stock has decreased gently and progressively; (2) the average number of errors per rat made by the individuals of the tank-trained stock has become much less than that provided by the individuals used as controls; (3) rats of the trained and control stocks respectively are to be distinguished one from the other by marked differences in the behaviour they exhibit during actual training whilst in the tank; and (4) during the preliminary 6 runs in the tank with alternating light and no shock, individuals of the two stocks are to be distinguished one from the other by their behaviour in relation to the light.

II. APPARATUS AND METHODS

In order that I might be in a position to examine these conclusions I have copied as far as possible McDougall's apparatus and methods. Since he had found that modifications and improvements in the construction of his tank had seriously influenced the scores of his rats, I tried to make a tank that, as far as I could judge from the descriptions given, was a fair copy of his latest edition. However, having seen it, McDougall tells me that our tanks do differ, and this has to be remembered when our results are compared.

My tank (Fig. 1), roughly square in outline with the corners of one side rounded off, is of sheet metal and measures $28\frac{1}{2}$ in. across, and $23\frac{1}{2}$ in. from the middle of the straight side to the middle of the curved one. It is 15 in. deep and is divided into three compartments by two incomplete partitions. The lateral compartments are $10\frac{1}{2}$ in. wide, and the middle one $7\frac{1}{2}$ in. The partitions are $14\frac{1}{2}$ in. long, and their free ends are curled outwards upon themselves in order that the rat may not be able to grip the edge. The depth of the water (kept at $60-62^{\circ}$ C.) is 9 in. In each of the lateral compartments on the straight wall of the tank there is a square hole just above the water level, and into it there juts an enclosed rocking platform. The rat, in leaving the water, clutches and tilts this platform, and in so doing completes an electric circuit. As the rat moves along the platform this returns to the level and the current is broken. In the movable roof of each lateral compartment there is a 5 candle-power electric light which is so shaded as to illuminate brightly the platform and also the whole of the lateral passage, so that the rat, about to leave the central blind compartment, can see which side is illuminated and which is dim.

My electric supply is taken from the main, 230 volts A.C., and passes through a neon lamp of 0.5 watt to be reduced to 0.002 amp. In view of the fact that McDougall had found that the number of errors made by the rats was fewer with a strong than with a weak shock, I wished particularly to ensure that my shock should be identical with his. But I could not do this, for he measures the strength of the current by its effect upon the rats: "the current is strong enough to tetanise the muscles of the rat's legs and hold him fast in as nearly as possible 50 per cent. of all contacts made by any batch of rats." He controls the duration of the shock by counting three slowly before breaking the current and releasing the rat. I tried by mechanical means to standardise



the duration and at the same time preserve the strength of the shock, but after many trials and failures I decided that though I standardised the strength and duration of the shock, I could not hope to standardise the rats, for not only do individuals differ among themselves in respect of their reactions (a shock that merely tickles one being sufficient to tetanise another), but the effect of the shock is determined very largely by the age and size of the rat, and especially by the particular way in which it makes contact with the platform. My shock is sufficient to "fix" the rat that grips the platform with his hands. I hold the rat there for 3 sec. and then cut off the current and so release the rat. But should a rat bump the platform with its nose, and particularly should it grip the platform with its teeth, unless the current is cut off immediately the rat is paralysed and usually does not recover. The strength of the shock is constant, but the duration varies from rat to rat and from time to time. By manipulating a switch I accommodate the rat that rushes the platform, the rat that bumps the platform with its nose or grips it with its teeth, and the rat that is so feeble that it takes a relatively long time to hoist itself out of the water. Agar (1935), who is also repeating this experiment, and whose first report has appeared since the first draft of this paper was written, has devised mechanical means of controlling both the intensity and the duration of the shock, and has eliminated the personal element. This, of course, is much to be desired, but unfortunately I cannot adopt his plan at this stage of my own experimentation. I must remain content with the knowledge that I myself have been trained along with the rats during the last 5 years, and that, as far as is possible, my manipulation of the switch has become automatic and standardised.

Obviously, if a considerable number of rats were electrocuted, a selective mortality might be operating and affecting the end-result of the experiment. But such injury only occurs during the very early days of training, and so it is most improbable that those which are hurt are only such as would have made high scores or low scores. McDougall has lost less than 2 per cent. from this cause, and I have lost only 8 rats among 2000+, and these all during the earlier years of the experiment; and so, in discussing our results, death from electrocution can safely be disregarded.

The tank itself rests on a table of such a height that the observer, seated, can, by looking over the lip of the tank, see the rat in the middle compartment and later swimming along the lateral passage toward the exit. The experiment is carried out in a darkened room: the only lights

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are those above the lateral passages in the tank and another on the switchboard.

When the litter is removed from its mother, each rat is ear-marked: the first male to be withdrawn from the cage becomes male No. 1 of that litter, the first female, female No. 1, and so on. At one time I thought that perhaps the rat that thus became No. 1 differed from the rest, that it was the first to be removed because of some peculiarity that was associated with quickness in learning. But examination of my own figures has shown me that such a suggestion is unsound, and so I have no comment to make upon McDougall's method of taking certain individuals from a litter at random.

In the beginning I adopted McDougall's plan and began the rat's training when it was 4 weeks old, but I was forced to the conclusion that my rats of this age could not tolerate 6 immersions in rapid succession. Moreover, with the shock that I was using, danger attended every trial. It may be that in Edinburgh the rat and its coat are not sufficiently well grown at 4 weeks. I decided to postpone the commencement of training until the rats reached 8 weeks of age. This difference in age between my rats and those of McDougall and of Agar at the beginning of training must be remembered when a comparison of our results is made. I have no reason to think that this makes any real difference to the end-result. I have trained individuals of small litters at 4 weeks and have compared their records with those of others trained at 8 weeks, and it would seem that the delay is attended by a somewhat higher average score in the case of my rats. In general, it appears that the smaller the rat the greater is its sensitiveness to the shock, and that the more severe the shock and the greater its duration, the lower is the score. In order to facilitate the rats' emergence from the water a submerged wire ladder is placed in front of each exit during the first 50 runs of the actual training period, and thereafter removed. The result of its removal is to increase the severity of the shock, for with the ladder in position, the rat is able to spring from it and pass rapidly over the electrified platform. In the absence of the ladder the rat must pull itself out of the water by gripping the platform itself. It was upon McDougall's advice that I introduced these ladders. He pointed out that in his tank the rat could rest upon a platform in front of the exit and take time to think things out. It could refuse to leave the tank by that route and re-enter the water and make its way to the alternative exit. But I have found it convenient to have movable ladders, for, after 50 runs or so, the rat that rests upon the ladder conveys no suggestion to me that it is

considering whether or not to leave the tank by that particular exit. It simply sits there, apparently very content to have removed most of itself from the water.

In the case of the earlier generations of my rats I adopted McDougall's plan of giving each rat 6 preliminary runs in the tank with the light alternating but without shock. McDougall had noted that "while the members of most litters go pretty evenly to both *A* and *B* (the right and left exits) before training, all the members of some litters and some members of others showed a strong bias to one side or to the other. It seemed that what might be called a right-handed or a left-handed tendency, or a tendency toward or away from the light, was innate in some strains, quite apart from any training of their ancestry." McDougall estimates that of his rats about 50 per cent. of all leave by the right or left platform irregularly, and about equally often; about 45 per cent. acquire at an early stage the habit of always leaving the tank by one and the same route and continue in this habit up to the time of learning; and that less than 5 per cent. at some stage of their training process, after turning right or left at random, acquire the habit of turning always to the illuminated side. These estimates relate to the actual training period when light and shock are alternating.

In view of these observations it seemed to me necessary to examine each rat before the light was introduced, for evidence of this right-hand and left-hand turning habit, and again with the light alternating but without shock, for evidence of the habit of going towards or away from the light as light. So each rat of the later generations has been given 50 runs in the tank with the light equal on both sides and without shock. The roofs of the lateral passages are removed and the room light immediately central to and above the tank is switched on. Thereafter, with the room darkened the light in the tank is alternated but the current is cut out for another 50 runs. These two phases of the preliminary training being completed, the third phase (alternating light plus shock) is then commenced. It is just as well that I did adopt this plan, for Agar reports that the great majority of his rats have shown a preference for the right-hand passage, and further, that they have a slight but unmistakable initial bias to the light. Again, these observations refer to the actual training period. It is to be noted that Agar derives his conclusions relating to the behaviour of his rats in respect of right- and left-handedness and of avoidance or otherwise of the light from figures provided during the actual training period when the light is associated with shock. It seems to me now, as it did when first I adopted this plan, that in any

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examination of handedness the experimentation must not be complicated by the presence of an alternating light, and especially by a combination of light and shock, and that in any examination of a bias to the light, this must not be associated with any other factor.

Each day and every day, for as long as is necessary, each rat is given 6 runs in the tank, one immediately after another. Agar's plan is somewhat different: each rat of a batch is placed in turn in the water, and the whole batch goes through the tank once before the first is placed therein again, so that the interval between two trials in the case of one and the same rat is much longer with Agar than with myself. This is not an important difference really, for my rats are twice as old as his when their training begins and therefore much more able to endure 6 immersions and exits in rapid succession. I adopted this plan because I found that by it I could recognise the behaviour pattern of any given rat much more clearly. Through time my assistant at the other end of the tank has developed an unvarying routine method of holding a rat and lowering it into the water at the far end of the middle blind compartment. But it really is of no consequence which way the rat is placed therein, for each rat, after it has completed its 100 preliminary runs, invariably displays a behaviour pattern which comes to be characteristic of that particular rat before leaving the central compartment to swim towards an exit. I agree with McDougall that after two or more rats have been in actual training for a fortnight or so it is possible to put them into the tank synchronously for the reason that the time spent in the middle compartment before moving towards an exit can differ markedly from rat to rat. If rats differing one from the other in respect of this habit are placed in the tank together they come out from the central passage separately and preserve the same order of leaving. I am satisfied that in the case of rats which after weeks of training have still not learnt, this procedure in no way disturbs each rat's demonstrated preference for one or the other exit. Agar regards the practice of having several rats in the tank at the same time as unsafe, since the behaviour of one rat might influence that of another. I use it only in the case of rats which after weeks of training show no signs of learning, and as soon as I recognise that one of the rats concerned is about to learn I run it through the tank alone. With this qualification I agree entirely with McDougall on this point.

Sooner or later, after a number of immersions and escapes, and after receiving a number of shocks (a number which, according to my own records, ranges from 0 to 307) every rat leaves the tank 12 times in

succession by the dim unshocked exit. Unlike McDougall, who has encountered rats "that would rather drown than leave the tank", I have never had a rat that did not ultimately complete its 6 daily runs and finally leave 12 times in succession by the safe route. No rat can learn in a day: this is important, for experience has shown that a rat about to learn is much more likely to make a mistake on the first than on subsequent immersions of a day's training.

Though, for purposes of comparison with McDougall's figures, it is necessary to carry on with the training of a rat, no matter how slow, until it has finally learnt, I am quite sure that by increasing the severity of the shock or by blocking up the lateral passage with a sheet of glass or metal it is possible to reduce very considerably the time required by the rat to learn.

My rats, like McDougall's and Agar's, were Wistar derivatives. They had their origin in 2 pairs imported directly from the Wistar Institute by my colleague, Dr Wiesner. The experiment started with 124 rats: 24 destined to become the ancestors of the experimental line, and 100 to form the first generation of controls. The experimental group was derived from 4 litters out of full sisters by the same male, their father. The controls consisted partly of the litter mates of these, but mainly of youngsters out of females related to the mothers of the experimental group and by the same sire. A further 100 related controls were set aside to form a breeding stock in order to provide control batches to be trained contemporaneously with each successive generation of the experimental line. After having satisfied myself that any possibility of communicability between parent and offspring and between trained and untrained could be disregarded, which I did by keeping the stocks in separate animal houses and by fostering controls on experimentals and *vice versa*, I kept both lines in the same house under identical conditions of husbandry which have never varied. The records of these stocks, when compared with those of the Institutional rat colony, permit me to hold the view that no nutritional deficiency and no disease of environmental origin is in any way responsible for the results I have obtained. This statement gains meaning perhaps in view of the unconfirmed observation of Tsai and Maurer (1930) that a vitamin-B depletion results in an increase in the incidence of left-handedness in the rat. During the course of the experiment microphthalmia made its appearance in the later generations of the experimentals and controls. One whole litter of 5 animals in the experimental line, every one of which was either completely eyeless or else had one blind or exceedingly small eye, was not trained. Four

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other individuals in the experimental line, and 3 in the control, were discarded for the same reason. Three experimental animals with the signs of "middle ear disease" and one with only one hindleg were likewise discarded. 19 rats (7 experimental and 12 control) have died from causes unknown shortly after having completed their training and without having reproduced.

It is quite impracticable to mate up all the individuals of a litter and of a generation; yet it is essential that any suggestion of selection should be avoided. I soon became aware that I must limit the number of rats in each generation, and that I must ensure that the slow learners of one generation, as well as the quick learners, should make their contribution in the form of offspring in the next. Since those which learn quickly are available for mating long before those of the same litter which learn slowly, nothing would have been easier than to have allowed a generation to be provided solely by the "quicks" of the previous generation. An examination of my pedigrees will show that at the beginning of this experiment one generation was being produced by one or two pairs of "quicks" of the previous generation, and that later, though few individuals of one generation are represented by progeny in the next, these include rats with low, median and high scores.

Since it seemed to me that the most serious defects in McDougall's procedures have been the neglect to maintain the records of the performance of every individual, to record all his animals on pedigree charts, and to use systematically an adequate number of controls, I arranged my own experimentation in ways which would remove from my own results flaws due to such deficiencies. The plan I devised happens to be that which in his third report McDougall states to be the most desirable, and that which, in the cases of his own rats, is now about to be adopted.

III. RESULTS

McDougall presents his results in the form of a table showing the average number of errors per rat and the number of errors made by the best and by the worst rat in each generation (Table I). The table relates to generations 13-34 only, for at the time when the first 12 generations were being trained the procedures had not been standardised and several modifications of the tank had been made, so that the results obtained could not be harmonised with those yielded by the 13th and subsequent generations. McDougall is of the opinion that the average error of the 1st generation, had it been recorded, would not have been less than 150

and that in all probability it would have been about 165+. The table shows that the highest arithmetical means representing the average number of errors are to be found at the top of the table, and the lowest at the bottom. It may well be that the differences in the arithmetical means do possess a real significance, but as they stand I do not think that they can support the superstructure of hypothesis that has been

TABLE I

Generation	No. of rats	Average no. of errors per rat	No. of errors made by	
			Best rat	Worst rat
1	—	165 +	—	—
13	23	68 +	30 (1)	90 +
14	10	80	42 (1)	102
15	10	70	39 (1)	96
16	5	73	39 (1)	88
17	11	46	9 (1)	147
18	22	62	15 (1)	142
19	15	47	12 (1)	100
21	34	37	9 (3)	74
22	16	36	6 (3)	89
23	26	25	3 (2)	71
24	14	33	10 (1)	62
25	18	38	14 (1)	78
26	23	43	9 (1)	75
27	32	54	12 (1)	96
28	17	44	13 (1)	90
29	20	50	18 (1)	105
30	11	20	3 (2)	56
31	38	40	3 (2)	100
32	42	17	3 (5)	70
33	24	33	2 (3)	73
34	34	36	2 (3)	88
Controls	140	122 + +	14	352

built upon them. The figures given in the table for the average score and for the scores of the best and worst rat of each batch cannot be accepted as a true measure of the distribution of these errors. Obviously, the average for any batch must be unduly affected by variation in the number of rats with scores of 100–300. In order to obtain a figure for the controls which McDougall used, since he himself has shown that in respect of the quality which was being examined strain differs from strain, it is permissible to select from his records such batches of rats as were related to his tank-trained stocks, and to neglect the rest (some 31 animals) which belonged to various and unrelated stocks. The controls then consist of 140 animals which give an average score of 122 + + with a range of 14–352.

Although in my opinion this method of presentation cannot convey all that is of significance, it will be convenient for purposes of com-

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parison if I give my results after the manner of McDougall. The actual figures for the experimental animals will be found in the pedigree charts and those for the controls in Table VII. In each generation the control and experimental batches are of the same age (within 7 days), and each control batch, consisting of more or less equal numbers of males and females, was trained contemporaneously with its own experimental batch and was derived from untrained litter mates of the animals used as controls in the preceding generation. The figures in black type indicate that the animal is a male.

In 1932 I presented a preliminary account of this experiment to the 6th International Congress of Genetics, and in so doing made a silly mistake that demands correction. I stated therein that the original 11 pairs of rats with which the experiment started (the remaining pair

TABLE II

Generation	Experimental				Control			
	No. of animals	Average score	Median	Range	No. of animals	Average score	Median	Range
I	22	31.31	32	5-47	100	18.08	16	0-81
II	39	23.86	18	0-89	50	42.74	19	0-194
III	80	20.51	17	1-92	50	61.24	38	0-291
IV	133	33.91	19	0-244	50	34.66	19	1-179
V	88	30.27	20	0-126	50	50.04	36	5-275
VI	43	52.34	49	4-152	50	48.44	34	4-123
VII	52	59.30	53	4-172	50	59.80	45	8-194
VIII	51	55.17	53	0-124	50	21.70	16	2-94
IX	67	61.64	55	0-181	50	32.50	16	2-162
X	60	43.85	39	0-152	50	69.12	58	4-201
XI	55	59.63	47	1-291	50	25.38	11	0-111
XII	65	50.38	42	0-283	50	23.22	13	2-101
XIII	133	41.36	23	0-212	50	34.90	19	0-121
XIV	160	52.75	36	0-307	50	52.86	26	4-291
XV	136	43.99	26	0-200	50	72.46	33	1-298
XVI	137	38.70	27	0-148	50	47.96	32	2-173
XVII	90	54.20	43	0-233	50	50.04	41	2-216
XVIII	38	22.23	15	0-97	114	26.39	12	0-144
	1449	43.39	—	0-307	1014	40.50	—	0-298

was electrocuted) gave an average score of 77.8 errors per rat. Actually, the figures I gave represented the average number of immersions and not the average number of shocks. I thought then that perhaps the length of time taken to learn might prove to be a more useful measure than the numbers of errors made. The average number of errors of generation I of the experimental line was 31.31, and not 77.8. It is seen that whereas McDougall's arithmetical means fell between 80 and 17, those of my experimental group range between 61.64 and 20.51, and those of the controls between 72.46 and 18.08. But whereas his highest

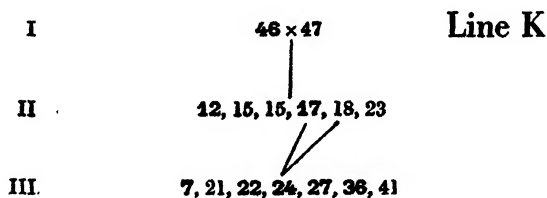
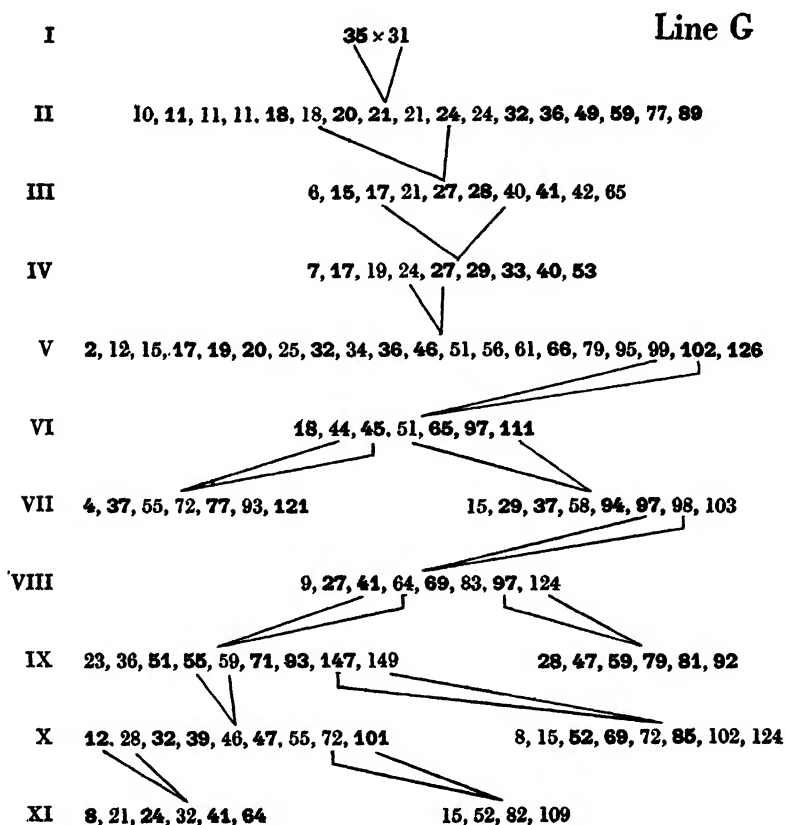
mean was given by his 14th generation and his lowest by his 32nd, *this fact permitting him to conclude that the later generations displayed a greater facility in learning than did the earlier*, the figures that I have so far obtained do not arrange themselves in any such order. In the experimental line my highest figure is in the middle of the table and the lowest is that given by the 3rd generation, whilst among the controls the lowest figure is that given by the very first batch. There is no suggestion of a gentle progressive decrease in the arithmetical mean in the case of my rats. There has been no marked decline in the average number of errors made by the best rat of each generation, in fact there could not be since one rat in my second generation of experimental rats and another in the first generation of controls had scores of 0. Neither has there been any decline in the number of errors made by the worst rat. Since McDougall's figures are not given in full I cannot carry a comparison between them and my own any further. I incline to the view that now that he has adopted methods of pedigreeing and recording similar to or identical with mine it is not improbable that in the figures that he will obtain during the next five years, these being exposed to the same methods of examination as are mine, there will be revealed the explanation of the disagreement that now exists between our results.

My figures further show that as far as my rats are concerned the average number of errors per rat made by the trained stock is not essentially different from that of the control. The average score of 1445 experimental rats is 43.39, that of 1014 controls 40.50. The range of the experimental group is 0-307, that of the control 0-298. It is of interest to compare these figures with those of McDougall (generations 13-34): best 2, worst 147; and of Agar: trained stock best 0, worst 142; control best 5, worst 143. But Agar's figure for the worst cannot really be compared with the others for the reason that he gives such rats as take a long time to learn special training which reduces their total number of errors.

Since in respect of arithmetical mean and range the two stocks and the later and earlier generations respectively are essentially similar, it follows that my figures do not suggest that there has been any unsuspected change in the conditions of the experiment or in the constitution of the stock during the period of the investigation. It is impossible for McDougall to make such a statement for the reason that he has not used adequate controls. This being so he is not justified, in my opinion, in relating the results he has obtained to any particular cause. Had he used controls properly and had observed this increased facility only in

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the trained stock his case would have indeed been strong. As it is, manifestly any unsuspected variation in the intensity of the shock, any pro-



gressive decline in general vigour consequent upon inbreeding (and he indicates that the reproductive rate of his present stock is causing anxiety), could possibly account for the improvement that he has observed. Further, in the absence of complete pedigrees, it is impossible

to know which of the animals comprising the earlier generations are represented in the later. Agar, in maintaining pedigrees, is in a position to know that 30 of the 34 parents of his 3rd generation are represented by progeny in the 5th generation. In my own case only 5 of the original 11 pairs are represented in generation II. Line K contributed nothing beyond generation III; Lines C and G came to an end in generation XI; so that generations XII–XVIII inclusive, for the time being at least, are composed of descendants of only 4 of the original 24 rats with which the experiment started, and these were the 4 with the lowest scores. I confess I have been surprised to see this state of affairs evolving, for the 12 original rats were out of an intensely inbred stock and, according to current genetical thought, ought to have been exceedingly similar one to the other in respect of genetic constitution. They were out of a stock that had undergone prolonged gene purgation, and yet the history of my stock reads like an experiment in inbreeding. There is a broad base of family lines and a narrow apex of two remaining lines. The reproductive rate falls, and line after line becomes extinct. Agar is wise in avoiding such close inbreeding as I have practised. However, the figures I have secured are of value in that they reveal the kind and degree of variability among the descendants of one or two pairs.

IV. HANDEDNESS

Before attempting to interpret further my own records relating to the number of errors, I propose to deal with the 3rd and 4th of McDougall's main conclusions: (3) that rats of the control and trained stocks respectively are to be distinguished readily one from the other by marked differences in the behaviour they exhibit in the tank during the actual training period, and (4) that during the preliminary period of the training individuals of the two stocks are to be distinguished one from the other in respect of their behaviour in relation to the light.

McDougall groups his rats into three classes in respect of their behaviour during the prediscriminatory period of their actual training. He estimates that about 50 per cent. of all his rats leave the tank by the right or left exit irregularly and about equally often; about 45 per cent. acquire at an early stage the habit of always leaving the tank by one and the same route, and continue in this habit up to the time of learning; and that less than 5 per cent. at some stage of the training process, after turning right or left at random, acquire the habit of turning always to the illuminated side.

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As I have already stated, because it seemed possible that a particular behaviour pattern of this kind might affect the score, I decided to test for handedness and for movement towards or away from the light before the shock was introduced. The graph (Fig. 2) gives the records of 568 rats of the experimental batch and of 500 controls in respect of handedness (light equal on both sides and constant, and no shock). It

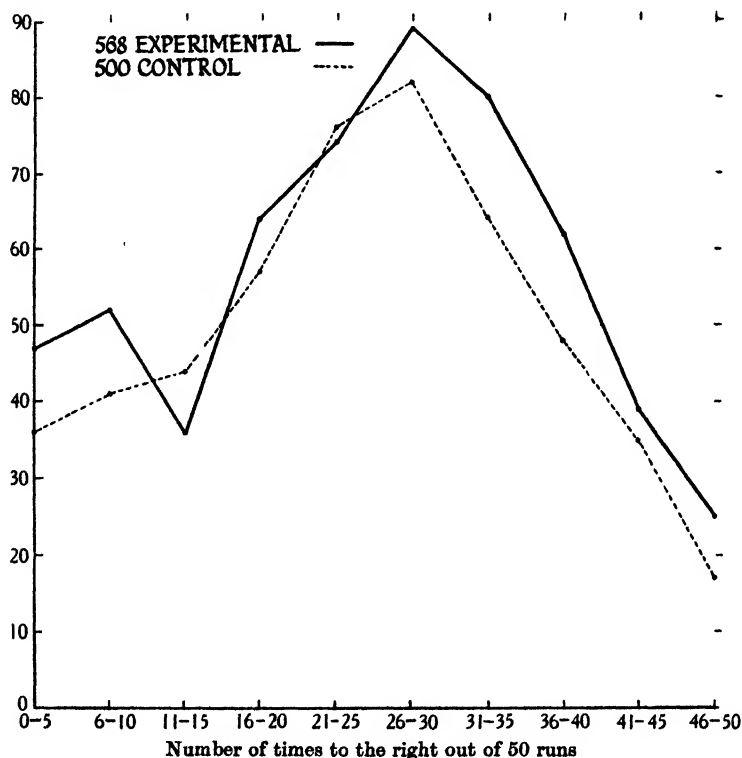


Fig. 2

is seen that there is no difference between the two groups, and that the graph does not even remotely resemble a chance distribution. There is a great excess of rats which tend to go habitually out of the tank by one route, the right or else the left, and there is a majority in favour of the right-hand turn. According to my records there are three easily distinguishable categories of rats; those that habitually make the right-hand turn (40 or more times to the right out of 50 runs), those that make the left-hand turn (10 and less times to the right), and those that turn

equally often to right and left (20-30 times to the right). In order to examine the effect of these habits upon the score I compiled Table III from the graph (Fig. 2). It will be seen that, of 1068 rats, 456 could not be accommodated by this classification and fell into the disregarded 11-20 and 31-40 times to the right groups. Of the remaining 612, 321 favoured the right- and left-hand turn equally often, whilst of the rest (291), 175 displayed the left-hand turn and 116 the right-hand turn habit. (The preponderance of the right-hand turn habit in the graph is due to the fact that the 31-40 group is much larger than the 11-20.) This table indicates that handedness in the first and preliminary phase of the training cannot be related to score. I certainly expected to reach a very different conclusion, for it seemed reasonable to assume that the rat with a "one-way habit" would encounter more difficulty in learning than the rat that turned one way as easily as the other, for the reason that his habit would have to be broken down. I still think that handedness is a factor which influences the score, but that its action is overshadowed by others which refer to the various types of reactions on the part of the rats to the light, and especially to the shock.

TABLE III

Times to the right	No. of rats		Average no. of errors		Range	
	Experi- mental	Control	Experi- mental	Control	Experi- mental	Control
(1) 0-10	98	77	46.7	42.7	0-203	0-195
(2) 21-30	163	158	46.6	51.6	0-307	4-132
(3) 41-50	64	52	45.3	52.8	0-133	0-116

These figures are to some extent in harmony with the conclusion of Peterson (1934) that right- and left-handedness, as demonstrated in his case by the method of holding food, occurs in about equal numbers in a rat population, that ambidexterity occurs much less frequently than either of the other conditions, and that handedness itself is an enduring and stable characteristic. I concur in his statement that it is not dependent upon the dominance of one eye over the other, for I have occluded each eye in turn without in any way affecting the habit. Peterson assumed that if one hand was favoured for eating it would be stronger than the other and would thus influence the route taken by the swimming rat. He therefore made use of McDougall's water tank to test this assumption only to find that the animals could not be classified into definite right- and left-handed groups, apparently for the reason that the procedure aroused emotional conditions which complicated his

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own investigation. However, his figures show that 6 rats given 6 trials a day for 7 days gave a total of 128 times to the right and 124 times to the left, and that one of the rats went only once to the right and 41 times to the left, another 37 times to the right and only 5 times to the left. I am sure that had he used more animals he would have found that they could in fact be classified in the same way as mine.

This right- and left-hand turn habit revealed in the first phase of the training which my rats have received (light equal on both sides and no shock) is not to be confused with the right- and left-hand turn habit displayed by the rat during the third phase (alternating light and shock). An examination of my records makes it abundantly clear that in about 90 per cent. of all cases, unless a rat learns within the first 4 or 5 days of its training, even though up to this point it has been going to right and left equally often, it settles down to a one-way habit, going continually either to the right or else to the left, and, having settled down so, it will continue to leave the tank by this one route for a period of time that may be anything from 2 days to 2 months, and will then with apparent abruptness, that at first surprises the observer, swim down the middle compartment and take the alternative route out of the tank. Of my rats which display this one-way habit in phase 3, approximately 60 per cent. take the right-hand turn and 40 per cent. the left. The behaviour of the rat is such as to permit one to think that after a few days of trying both exits and getting shocks at both, it concludes that it may as well stick to one route. Thereafter it seems completely to have forgotten the existence of the other. But if one has been noting the time spent by the rat in the middle compartment before making for the chosen exit, it becomes clear that a day or two before the rat is to begin to learn, this time is longer when the chosen exit is lit (and shocked) than when it is dark. A difference of 2-5 sec. is not uncommon, and when looked for is easily recognised. At this time, too, if as the rat is turning into the lateral passage the light is switched off and on rapidly, it will often turn away and leave the tank by the alternative exit, a procedure which has no effect unless the rat is on the point of learning. If that lateral passage down which, according to its habit, a rat will inevitably pass is blocked, the rat can be taught that there is an alternative route out of the water long before, under the ordinary conditions of the experiment, this would have been realised. Also, if a rat has been taking the right-hand turn, for example, for 150 or 200 times without a break, it can, with practice, be shaken off the electrified platform by manipulation of the switch, and if each time the rat approaches the platform the current

is switched on and off rapidly and the exit thus denied to it, sooner or later the rat will turn away and swim to the alternative exit. A few treatments will result in the rat learning after having made some 3-6 additional errors. Undoubtedly fatigue and irritation on my part have reduced in this way several scores of 250+ to scores of about 120-150. Agar has deliberately adopted a similar plan of giving special training to all his rats that have not learnt after 302 trials. It seems to me, therefore, that when McDougall states that about 50 per cent. of his rats turn right and left equally often, and that 45 per cent. persist in going either to the right or else to the left, he is merely stating in other words that about half of his rats learn quickly and the other half learn slowly, for it is the rat that learns slowly that exhibits the one-way habit during the actual training period.

Agar has observed that out of 323 rats of his generations 2-5, 212 formed a right-hand habit and 44 a left-hand habit, whilst 67 failed to do so. He states that the latter were mostly rats that learnt quickly, and quite rightly argues that early learning and failure to form the habit are undoubtedly causally connected. But he concludes that rats which do not quickly form the right-hand or left-hand habit tend to learn much sooner than those that do. My interpretation is exactly the opposite one: I hold the view that it is because a rat does not learn quickly that it develops the one-way habit. The situation as I see it is as follows: the rat is set the task of forming an association between light and shock but fails to do so so long as it must take into account both exits. It settles down to leaving the tank by one route. Later there comes into being an appreciation of the difference in experience when the chosen exit is illuminated and when it is not. The time spent in the middle compartment before leaving becomes prolonged when the light is on the chosen side, and shortened when it is not. Later still the memory of the alternative exit is rekindled or perhaps the rat begins to observe that when one exit is illuminated the other is dim, and then hesitation is shown at the entrance of the illuminated passage and the rat will swerve away toward the opposite exit. I agree with Agar that the extent of the swerve may make all the difference to the ultimate score, for a wide swerve can carry the rat right round the tank into the dark lateral passage, whereas a narrower swerve can find the rat once more back in the central compartment so that nothing really has been learnt.

But dozens of my record cards offer evidence which makes it difficult for me to agree with Agar's view that it is because a rat does not display during the earliest days of its training the one-way habit that it learns

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quickly. These records relate to rats which do not display the one-way habit until they have been irregularly alternating for 100-150 and more trials, exhibiting a behaviour pattern that forces me to the conclusion that thought is in no way determining their choice. My view is that as a general rule every rat that does not learn quickly (with a score of 20 or under for example) sooner or later develops the habit of persistently going either to the right or else to the left, and that the actual score in these cases is determined by the length of time this one-way habit persists. It is to be noted that though it is common for a one-way habit displayed during phase 1 of the preliminary training to be prolonged into phase 3, this is not always so, for if a rat with such a habit in phase 1 is to learn with a score of 2-5, obviously there will be no opportunity for such a habit to be displayed. If the rat is to make a score of 150, on the other hand, it will sooner or later display the one-way habit in phase 3, and in the majority of such cases, though not in all, the chosen side in phase 3 is the same as that in phase 1.

I agree with Agar that McDougall is mistaken in assuming that in the case of these animals with a one-way habit in phase 3 the rat is required to discriminate between a lit exit on one side and a dim exit on the other. It is quite clear that the first discrimination in these cases is between the occasion when the chosen side is illuminated and shocked and when it is not. But this refers only to rats which are slow learners and display the one-way habit; these first discriminate between light and no light on one and the same side, and only then achieve the discrimination between the one side that is illuminated and the other side which is dark. It does not apply to those rats that learn quickly with scores of 10 and under, for their behaviour indicates clearly that they are aware that there are two ways out of the tank.

V. REACTIONS TO THE LIGHT

Because McDougall had estimated that some 5 per cent. of his rats acquired the habit of turning always to the illuminated side, because he had concluded that during the preliminary 6 runs with alternating light and no shock, individuals of the trained stock tended on the whole to show a slight preference for the dim route whereas the controls showed a slight but decided preference for the bright route, because he had had rats in his first experiment which learnt without making a single error, though he regarded it as a happy chance that the rats had done so, and especially because in my own first batch of controls I had had one rat

which made a score of 0, I decided that I must test every one of my rats for photophobia. Phase 2 of my preliminary training consists of 50 runs with alternating light and no shock. Agar also has encountered one rat that made not a single error, but because not one of its ancestors or descendants in the next generation had a similar score, he decided that this avoidance of the light was not due to genetic factors.

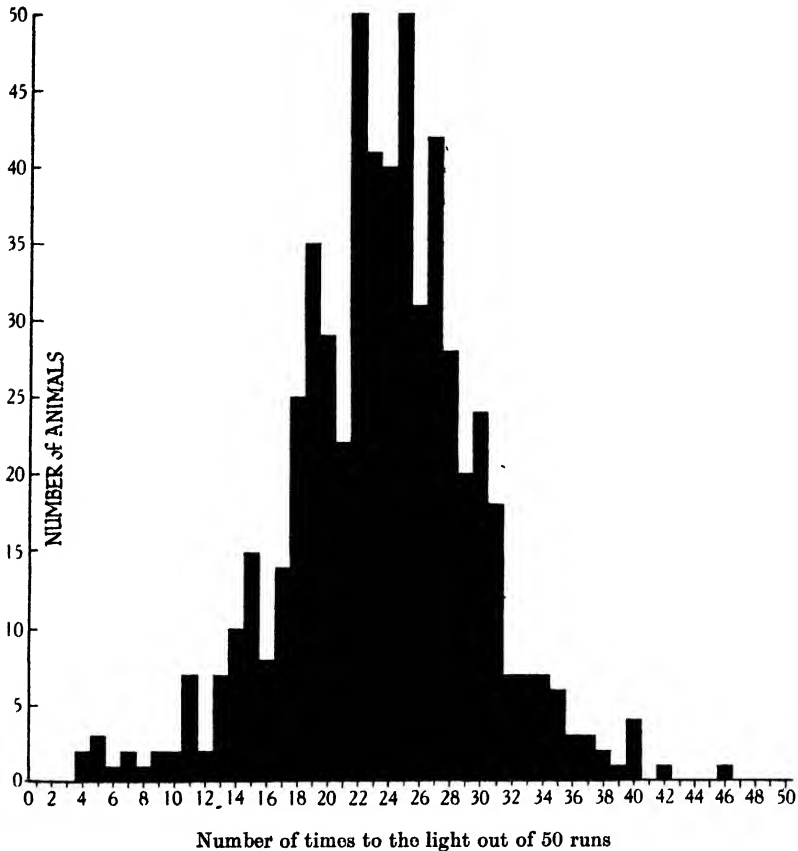


Fig. 3

The diagram (Fig. 3) depicts the records of a group of 573 experimental animals. Since those of 500 controls give an exact replica of this, I do not show it. Experimental and control lines are not to be distinguished. Clearly the distribution here is not determined by chance. That this is so is shown even more clearly in Table IV. Assuming that it is a matter of chance whether a rat goes towards the light or

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away from it, from the binomial distribution, the points beyond which 5 per cent., 10 per cent., or any other proportion of the observations should lie, can readily be calculated. The table has been constructed to show the portions of the range within which each succeeding 10 per cent. of the observations should lie (except that the end classes have been divided into two).

TABLE IV

Range	Expected number	Observed number	χ^2
0 -19.18	29	125	317.8
19.18-20.47	29	39	3.4
20.47-22.02	57	49	1.1
22.02-23.14	57	51	0.6
23.14-24.10	57	38	6.3
24.10-25.00	57	41	4.5
25.00-25.90	57	37	7.0
25.90-26.85	57	34	9.3
26.85-27.97	57	40	5.1
27.97-29.53	57	36	7.7
29.53-30.81	29	29	0.0
30.81-upwards	29	54	21.6
	—	573	384.4

Manifestly, this distribution is not one governed by chance. There is a significant discrepancy in the first and last of the classes, especially in the first, whilst the second is also similarly affected. There are very many more rats going to the light less than 20 times out of the 50 runs than would be expected. The chance of getting a rat that goes 12 times or less to the light is only 1 in 1000; yet out of 573 rats I have no less than 22 which went to the light 12 times or less. There are in my stock rats which avoid the light as light. It follows that such rats must be greatly advantaged under the conditions of the experiment since in avoiding the light they also avoid the shock.

Actually in the whole 2459 rats so far trained there have been 29 experimental and 10 control which never received a single shock for the reason that on each run of the first 12 of phase 3 they went to the dim and safe exit. 11 of these are included among the 573 rats in Table IV. When their records are examined it is found that the rat which makes a score of 0 in phase 3 has, during the later part of phase 2 of its training, been consistently avoiding the light. It is because they carry over this habit into phase 3 that such rats "learn" without getting a single shock. One rat of my control stock did not go to the light even once during phase 2, and ended its training with a zero score. Usually, however, such rats only begin habitually to avoid the light in the latter part of phase 2, the last half or third. As I have stated, the change from phase 1

to phase 2 of the preliminary training is usually attended by a disturbance in the behaviour of the rat. This lasts over 1-4 days usually, and thereafter the rat settles down again to its old habit or else to a new one. If a rat makes a score of 10 times or less to the light in phase 2 (and these all in the first half) the observer is justified in expecting that the rat will learn without making a single error. But this does not always happen. The rat may leave the tank 6 times in succession by the dim route on the first day of phase 3, but on the first run of the second day it may go to the light and get a shock. If the reaction is severe, for the remaining runs it may rush squealing, splashing, swimming perpendicularly in the water, spend its energy in the hopeless task of climbing the smooth walls, in fact may do everything that must make considered choice impossible. Until it behaves quietly again it continues to add to its score, and not until it does behave quietly can it learn. I have had rats with scores of 100 and more which might, I think, have learnt with 0 had they escaped the shock on the first run of the second day. Undoubtedly just as the one-way habit can be overcome by denying the chosen passage to the rat, so also can this deliberate avoidance of the light be overwhelmed by the strength of a rat's reaction to an electric shock.

The difficulty of demonstrating a habit is illustrated by the results of the following tests which I made with some of these photophobic rats. For example, in the last 29 runs of phase 2, a certain rat had repeatedly left the tank by the dim route. The light was then kept permanently at the right exit. But the rat continued to alternate. After some 12 runs, however, as if at length noticing that the conditions had changed, it began to hesitate, and then always left the tank by the dim exit on the left. This continued for 2 days and then the light was switched over and kept permanently on the left. But the rat continued to go to the left for 12-15 runs, then began to hesitate and later turned away, always to leave by the dim right exit. This behaviour is vastly intriguing. It would appear that when faced with the task of making a decision of this kind, the rat brings thought to bear upon the problem and solves it, the decision giving expression to a preference. The decision having been made, thought, no longer required, is banished and a habit is assumed. The rat having decided to alternate, alternates, and this habit carries it past the point where the conditions are changed. It carries it 2-3 days beyond this point. Then thought enters once more, and again the same series of events is launched. It becomes a matter of some difficulty to demonstrate that any given rat is definitely photophobic. Nevertheless

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the difference between the behaviour pattern during phase 2 of these rats and of the rest is such as to make it quite certain that they are avoiding the light as light to begin with and thereafter continue to alternate as a habit.

I am of the opinion that a rat can display this alternating habit for other (and to me unknown) reasons. It has been shown that no less than 125 out of 573 rats went to the light less than 20 times out of 50 runs and that 54 went to the light 30–81 times and over. The expected numbers in both classes is 29. I am quite sure that a considerable proportion of the 125 rats were not deliberately avoiding the light as light, and that all the 54 rats were deliberately going towards it. There are rats, undoubtedly, which quickly develop the habit of leaving the tank first by one route and then by the other. They march in step with the alternating light and shock for a time, getting shock after shock, then go twice to the same exit and thereafter alternate again, so avoiding shocks, then go twice to the same exit and get into step with the shock again. Such a rat at irregular intervals leaves the tank 7 or 8 times in succession by the dim route, but the observer is not deceived into thinking that the rat is likely to learn. But it occasionally happens that such a rat does learn in the sense that it makes 12 successive safe escapes. But it has not learnt in the sense of having achieved discrimination, as can be shown by continuing the training process. Such a rat will certainly begin to pile up another score, whereas the rat that has really learnt only rarely makes a mistake during the 3 months subsequent to its completion of training. But for the great majority of the rats McDougall's test is adequate, and the rat that learns without mastering the task is not very common. I suspect that the whole of the excess in the 30–81 upwards class and the same number in the 125 of the first class belong to this alternating category. This would still leave some 70 animals in the 0–20 times to the light class, the behaviour of which I think is to be explained by reference to an initial deliberate and conscious avoidance of light as light.

The average score of a given batch of rats must necessarily be influenced greatly by the number of such photophobic rats within it, and so in any experiment of this kind it is necessary to include a test for photophobia. It is incumbent upon McDougall to show that the explanation of his observation that his tank-trained stock tend to avoid the bright gangway whereas his controls tend to prefer it is other than that through unconscious selection there are now many more photophobic animals among his trained stock than among his controls. Such

an explanation would at once accommodate the observation that this preference made a sudden appearance, so sudden indeed as to suggest to McDougall that a mutation had appeared, having been induced by the training process in some earlier generation.

I very much doubt, however, that McDougall is warranted in basing any conclusion whatsoever on the behaviour of the rats during 6 runs with alternating light and no shock, especially if these are the first runs that the rats have ever had. For myself, I am satisfied that before the rat's reactions to light are tested, it must first have been accustomed to the tank with the light equal on both sides. I have hundreds of records in which a suggestion relating to behaviour conveyed by the earlier entries of each of the phases of training is completely contradicted by the behaviour of the rat during the rest of the phase. This being so, I am unwilling to accept McDougall's fourth main conclusion.

Agar states that his rats show a slight but unmistakable initial bias to the light during the actual training (my phase 3). Frankly I do not think that his actual observations warrant such a conclusion. It is during the first few days of the actual training that a batch of rats, which does not include a great preponderance of individuals that are to learn with very few errors, will necessarily make most mistakes, and in so doing will of course go more often to the light than away from it. Those that do not learn quickly tend to develop a one-way habit and this means that they cannot make more than three mistakes and go three times to the light more than three times a day, whereas an "alternating" rat can make more and go more often. I do not think that whilst the light is associated with the shock it is possible to relate the behaviour of the rats to one of these alone, since both light and shock provoke reactions which can disturb the behaviour displayed prior to their incoming. This is demonstrated by the increase in the frequency of defaecation whilst in the tank that occurs at the beginning of each phase of training, and especially during the first week of phase 3. It is not improbable that frequency of defaecation under these conditions might form a measure of the emotional behaviour of the rat, for those that defaecate every time they approach the exit are those that can be regarded as the most excitable. Surely it would have been equally correct or incorrect for Agar to have stated that his rats showed a slight but unmistakable initial bias to the shock. In my opinion his figures which show that his rats during the first 3 days of their training went to the light 2139 times instead of the 1968 times which would have been expected, had the choice of going to the light or away from it been random, simply illus-

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trate the fact that the majority of rats are more exploratory and make most errors per unit of time during the first week of their training. At this time and under these conditions it is the shock and not the light that is conditioning behaviour.

VI. TIME IN THE TANK

McDougall's remaining conclusion that rats of the two stocks, trained and control, are to be distinguished by characteristic differences in their behaviour in the tank is, in my opinion, insecurely based since he has not used sufficient controls and has not used them properly. He is satisfied that the rats of the trained stock tend to stay in the water for a longer period of time before making for one or other of the exits than do the controls; that the trained stock rat typically is more cautious, more hesitant; and the implication is that it is possible to state whether any given rat is a control or an experimental after having seen it in the water during the first few days of training.

Like McDougall I have kept records of the time spent in the water by groups of the two stocks. The average time per rat for a representative group of 500 controls and 500 experimentals is roughly 3 min. per rat per day of 6 runs. There is no difference between the two stocks. But this figure possesses very little meaning, save that it indicates the total number of hours the observer spends in the dark during the course of a year; actually the time spent in the water varies markedly from rat to rat, and in the case of one and the same rat, according to the stage of its training. During the first and second phases of my training it is usual for each rat to take $1\frac{1}{2}$ min. for the 6 runs, but the pace is much slower during the first day of each phase than subsequently. On the first day of the third phase when the shock is introduced the majority of rats, after their first shock, will remain in the water for some $1\frac{1}{2}$ min. before beginning to move towards the exit. (Quite a number react in exactly the opposite way, rushing wildly at the platform, leaping over it and coming out of the tank like a ball from a bat.) For the next 2 days the time in the water is considerably lengthened and thereafter speeds up again to slow down once more as the point of discrimination is approached. Usually a rat stays longer in the water on its first immersion of the day than subsequently. The following sequence is not uncommon: $1\frac{1}{2}$ min., 1 min., 30 sec., 15 sec., 10 sec., 10 sec. But no day is complete without the rat that stays 5–10 min. on its first immersion. I have had them, both experimental and control, stay as long as 35 min. But during

the last 2 years these have not caused me any distress for I just leave them to soak in the middle compartment and carry on with the training of others. Usually there is in training a rat that stays in the water whilst 6 or 8 others pass through their daily training, and their activities in no way affect the behaviour of the laggard.

It is really astonishing to observe the display of a constant and characteristic behaviour pattern in the tank. The rats exhibit amongst themselves appreciable differences in the time spent in the middle compartment; in the method of swimming, some floating, some gently paddling to produce a quiet, rhythmical scratching on the wall of the tank; in the method of turning to face the open end of the middle compartment, some always pushing off with the hindlegs from one or other partition; in the method of approaching the ladder, some directly from the front, others always from one side; in raising themselves out of the water, some using only their forelegs, others mainly with their hindlegs; in their behaviour after emergence, some quickly peering about, others obviously excited and nervous. These and many other idiosyncracies are to be observed, but none of them is characteristic of one or the other group. It is possible to identify an individual in a small batch by reference to its behaviour certainly, but I am quite unable to distinguish an experimental rat from a control in this way.

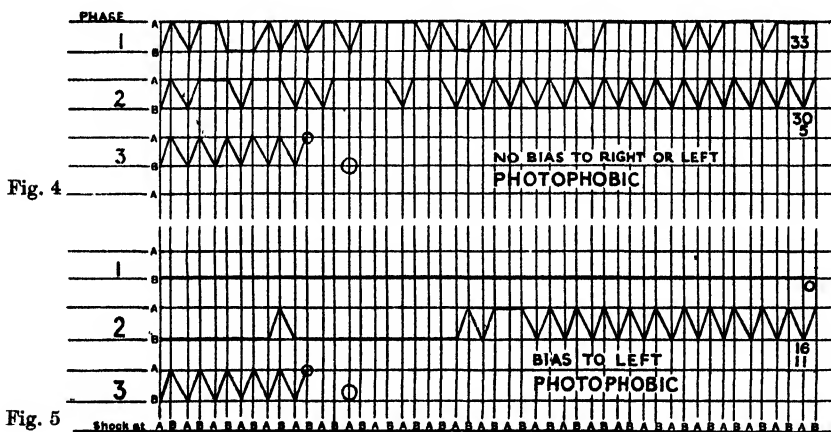
VII. EXAMPLES OF RECORD CARDS

My procedure, results and conclusions can best be demonstrated by the presentation of copies of representative record cards. Each card can accommodate the records of 500 trials (350 on the front, 150 on the back). *A*=the right exit, *B*=the left. The horizontal lines are blue, the *A* vertical lines are red, the *B* vertical lines are blue. The first row (*AB*) is used for the records of the 50 runs of phase 1, equal light on both sides and no shock; the second row for the 50 runs of phase 2, alternating light and no shock; whilst the remaining rows are used for phase 3, alternating light and shock. If, on its first run of phase 1, the rat goes to the right, a dot is put where the vertical red *A* line cuts the horizontal *A* line; if it goes to the left, the dot is put at the junction of the vertical red *A* line and the *B* horizontal line. On the second occasion, if the rat goes to the left, then the dot is placed at the junction of the two *B* lines; if it goes to *A*, then it is put where the vertical blue *B* line intersects the horizontal *A* line. At the end of the 6 runs the dots are joined by lines. On the 8th day 8 runs instead of 6 are given

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to bring the total to 50. The number of times to the right out of the 50 is then written down at the end of the line. At the end of phase 2 the total number of times to the right, and also that of the number of times to the light are recorded. In phase 3 the total number of errors in each row is recorded in the column on the left and these are added together to give the final score. The diagram provided by the record card gives an immediate appreciation of the rat's behaviour.

Fig. 4 is a copy of the record of a rat which, having no bias in respect of handedness, and being photophobic, learnt with a score of 0. It went to the right 33 times out of 50 in phase 1, and 30 times out of 50 in



phase 2. But in this second phase it went to the light only 5 times out of 50, and for the last 29 times invariably went to the dim exit. This habit persisted into phase 3 and the rat learnt without receiving a single shock.

Fig. 5 is the record of a rat with a strong bias to the left; it did not go once to the right in phase 1. During the first half of phase 2 this habit continued, and it went to the light 11 times. But during the second half of this phase, the rat for 23 times in succession went to the dim exit. This new habit was carried over into phase 3 and the rat learnt with a score of 0.

Fig. 6 is the record showing the astonishing persistence of habit in a photophobic rat after the conditions of the test have been changed. Shock is not involved in this case. After the rat had left the tank on 26 successive occasions by the dim route, the light alternating, the light was kept at A. The rat continued to alternate for a further 12 runs and

thereafter settled down to leave the tank always by the dim *B* route. After it had continued to do this for 18 consecutive runs the light was kept at *B*. The rat continued to leave by the *B* route for a further 9 runs and then changed over to *A*. After it had done so for 21 runs the light

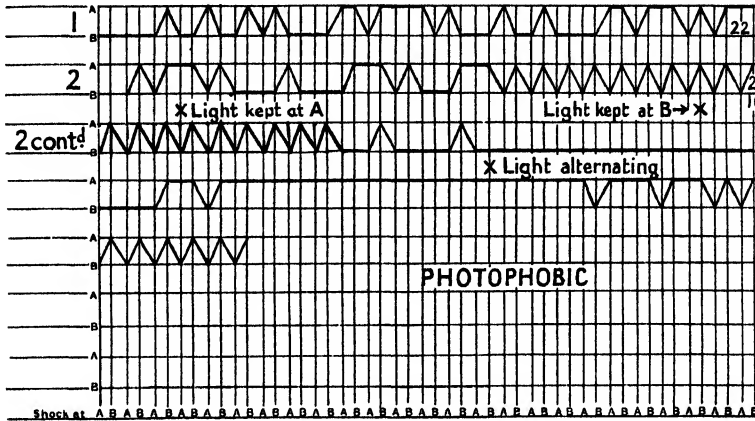


Fig. 6

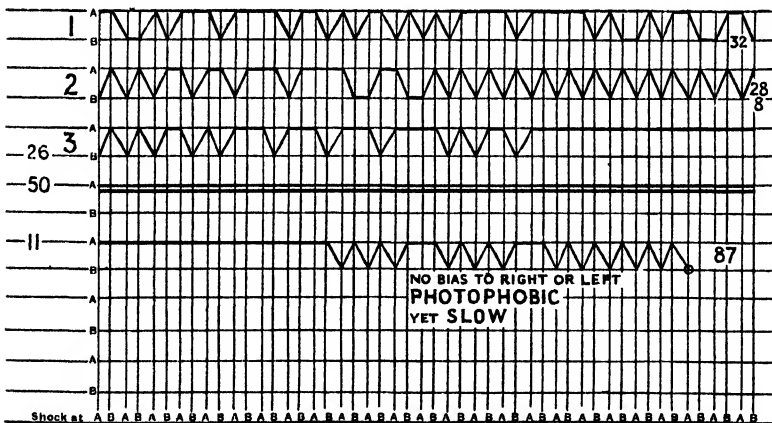


Fig. 7

was alternated again, but the rat kept to the *A* route for 7 further runs before beginning to alternate once more and to leave by the dim route.

Fig. 7 shows the record of a rat which according to the evidence of phase 2 is photophobic and which I expected to learn without an error. It received a shock on the first run of the second day, reacted strongly,

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its alternating habit was disturbed and the rat received many shocks and then settled down to the right-hand turn for 137 runs before it began to learn.

Fig. 8 shows a rat with a strong left-hand bias. It went to the right

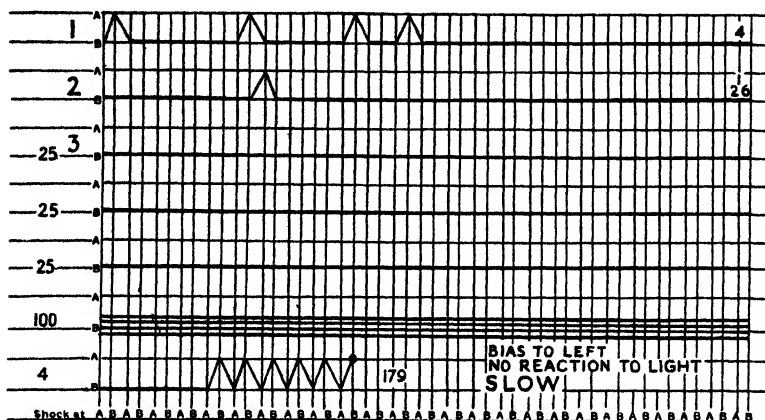


Fig. 8

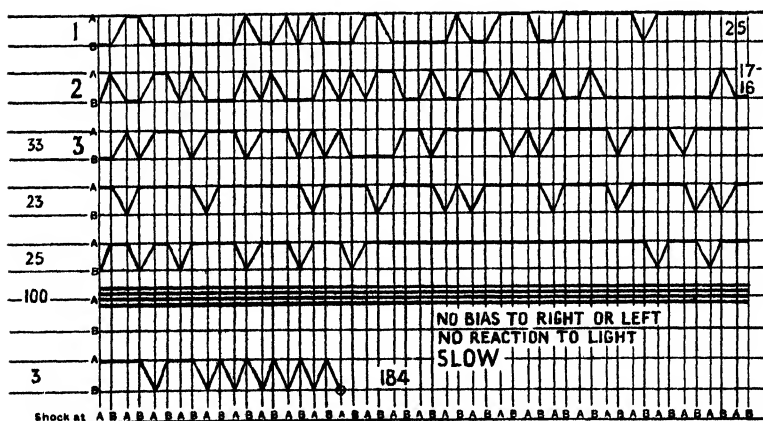


Fig. 9

only 4 out of 50 times in phase 1, once to the right and 26 times to the light in phase 2, and continued to go to the left in phase 3 for 359 consecutive runs. The apparent abruptness of the attainment of discrimination is clearly illustrated in this record.

Fig. 9 shows a rat which went to right and left equally in phase 1,

Fig. 10 is the record of a rat which exhibited the right-hand bias strongly and this was not disturbed by the alternating light in phase 2 save in the beginning. (It is seen that the subsequent behaviour of this rat could not have been predicted from the performance during the first 6 runs.) It went to the light 26 times. In phase 3 on the first day it got



Fig. 11 shows a rat with no obvious bias and no regard for the light that is quick to achieve discrimination.

So, according to my records, there are rats with a bias to one side or to the other, and rats without this bias; there are rats that avoid the light as light and rats which do not react to it; and there are rats which are quick to achieve discrimination and rats which are slow. There are rats with a one-way bias which are quick and others which are slow. Similarly there are quick rats and slow rats without this bias. I assume that there are photophobic rats which are also quick and others which are slow, and that there are quick and slow rats which are not photophobic. These differences themselves are more than sufficient, singly and

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in combination, to make their analysis extremely difficult. But, in addition to these factors, which must largely determine the actual score, there are others which are to be described only vaguely. A runt, the feeble, poorly developed individual, learns quickly for the reason, I assume, that the punishment it receives is more severe. If this is so, then, as a general rule, the more vigorous the rat the higher the score may be expected to be, and so in comparing the performances of the later with the earlier generations it becomes necessary to show that in respect of general vigour there has been no steady deterioration. The members of a small litter are at a given age better grown than are those of a large litter, and so there is a tendency for the members of a large litter to give lower scores than those of small litters. (Agar wisely reduces his large litters: I have not done so.) Some rats are much more excitable than others, and these give themselves scanty opportunity for considering the problem set; they begin to swim whilst still being lowered into the tank, and immediately rush squealing down the middle compartment. They are handicapped in comparison with the quiet gentle creature that paddles about seeming to explore the tank. So that general vigour, timidity, excitability, even the habit of swimming near the outer wall of the tank, are factors which also enter and necessarily complicate the analysis.

VIII. MY RATS TRAINED BY McDOUGALL

If such peculiar idiosyncracies affect the results of an experiment such as this, differing proportions of the various types must make comparison between McDougall's, Agar's and my figures somewhat difficult. I do not think for one moment that our personal preferences could in any way influence the performance of the rats in the tank, but our tanks are different, our procedures are different, as are also the intensity and duration of the shock. But such differences are not really important since each of us has rats which under the conditions which obtain learn quickly and others that learn slowly, and the problem that each of us is investigating is the same: each is studying the question as to whether or not a high average score of early generations is converted into a low average score in later generations, and, if so, by what means this is brought about. But if many constitutional factors, general vigour, excitability and so on, which have nothing to do directly with the ability to achieve discrimination but which affect the speed with which this is acquired, are concerned in the experiment, manifestly the results of the three of us can be hopefully compared only if our rat stocks are of

similar constitution. If, for example, I had started with a number of photophobic runts, and if photophobia and physical feebleness "bred true", I should have got and have continued to get generation after generation of exceedingly low-scoring animals, and at the end I should not have been in a position to comment upon McDougall's conclusions. As it happens, however, all three of us have been using Wistar rats, and as the result of the genetic teaching and skilled advertisement, we, I suppose, are prepared to assume that, because each of us got our foundation stock from this same source, our rats must therefore be genetically similar if not identical. But do Wistar rats remain Wistar rats when they are born and bred in Durham, Melbourne and Edinburgh? I know that my rats no longer resemble their ancestors in respect of many of the more easily measurable qualities (Hain, 1934). I have to assume that selection of a kind has always been operating in my own rattery. I have always deliberately been selecting rats that flourished best in the conditions that obtain here, and I have inbred these most intensely. I think it would be found on examination that in respect of growth rate, litter size, age at death, incidence of abnormalities, my rats differ from those of McDougall and Agar, and certainly some of these are genetic characters. If so, then it would not be surprising, if any of the factors contributing to the achievement of discrimination in the tank were also genetic, to find that my stock is now more or less different from the other two. Obviously, there could have been no selection in respect of such factors before the experiment started, for their presence is to be recognised only by the complicated test in the tank.

Fortunately it is possible to examine the validity of this suggestion. In 1931 McDougall took back to Durham with him 12 of my rats whilst I kept their litter mates and trained them here. The rats that I gave him were out of my generation V, and those that I retained became the parents of my generation VI. At Durham these rats and their descendants in the hands of McDougall gave the following scores:

TABLE V

Generation	No. of animals	Average score	Range
I	11	188	16-282
II	20	78	15-315
III	14	63	10-150
IV	16	68	9-174
V	12	48	18- 75
VI	19	61	14-203
VII	21	46	4- 93

McDougall is inclined to regard these figures as supporting his own conclusions. It is seen that an arithmetical mean of 188 in the first

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generation is transformed into one of 46 in the seventh. But I am not willing to agree, for in the case of such small batches the average is disturbed too profoundly by the presence therein of one or more animals with very high scores. My unwillingness is reinforced by the figures I myself obtained from their litter mates. These are presented in the manner of McDougall (see generations VI–XI inclusive, Table II). It is seen that I have many more animals (328 against 113), and that the range is practically as wide (0–291 instead of 4–315). If the first two generations of my rats in Durham are disregarded, and it is surely fair to do this for the conditions there must necessarily be different from those in Edinburgh and rats undoubtedly require a little time to become acclimatised, then there is no difference whatsoever between the actual figures McDougall got and mine. This being so, I incline to the view that his own rats do not differ in any significant way from my own, and that the difference in our conclusions must refer, not to ourselves, not to our tanks or our procedures, not even to real differences in the results we have obtained, but to differences in our methods of selecting the parents of the succeeding generations and of recording, and especially in our methods of controlling the experiment.

IX. ANALYSIS OF PEDIGREES

In an attempt to determine whether or not I was justified in my strong impression that this quality that is represented by the score is in whole or part genetic, I divided my experimental rats into 9 classes with scores in geometrical progression, disregarding all rats with a score of 0, for the reason that photophobia seemed to be a quality to be examined separately, and then plotted the frequency distribution of the offspring for different parental matings. It was found that when both parental scores are low (≤ 17 –32, Class V) the offspring distributions are very varied and have no obvious common property save that their peaks (also their means and medians) are all ≤ 33 –64 (Class VI), whereas when both parental scores are high (≥ 33 –64, Class VI) the distributions are all very similar in shape, having a high peak in Class VII (65–128). Consequently the collective distribution of all the offspring of parents with low scores shows a wide dispersion, whereas that of offspring of parents with high scores has a high peak and a narrow dispersion.

These observations suggest that among these rats there are two main classes, the “quick” and the “slow”, and that in a general way “quickness” behaves as a dominant in relation to “slowness”. If this were so, then Classes VI–IX (33–257+) should consist largely of pure recessives,

slows, and this would account for the varied pattern of the distribution of the scores of offspring of parents with low scores and for the relative constancy of the distribution of the scores of offspring of parents with high scores.

It has to be acknowledged that this suggestion of a single main factor pair (or of some system of multifactor inheritance giving similar results), though satisfactory up to a point, does not accommodate the fact that a considerable number of "quicks" appear among the offspring of slow \times slow matings. It is also in conflict with the results of McDougall's subsidiary experiment in which training was combined with deliberate adverse selection (breeding consistently from the slowest in each successive generation) and with favourable selection (breeding consistently from the quickest). In the case of adverse selection McDougall got remarkable improvement after 14 generations, whereas with favourable selection no such results were obtained. He regards this ineffectiveness of adverse selection as the strongest possible corroboration of his main conclusions.

An examination of my pedigrees will show that I too have contemporaneously been practising selection in both directions, that my line *A* is now to be distinguished as a "quick" strain, being relatively fixed and yielding proportions of quick and slow offspring in matings of quick \times quick, quick \times slow, and slow \times slow which differ significantly from the rest, and that I have failed to fix to the same degree a strain of "slows". But surely there is a very simple explanation. It is far more difficult, in my opinion, for a constitutionally "slow" rat to pass through the test with a low score than it is for a constitutionally "quick" rat to put up a high score. Quite a number of happenings, casual or accidental, can convert a potentially low score into a high one, and so the "slows" come to include a number of "quicks". For this reason I would expect the quicks to be relatively uniform and the slows heterogeneous. Thus if "quickness" and "slowness" were genetic characters, in whole or part, and polygenic in nature, and if selection of the worst implied the selection of individuals of different genotypes whilst selection of the best meant selection of a more uniform genotype, I should not expect favourable selection to be followed by a drop in the arithmetical mean, but I should expect that this might easily happen if adverse selection were practised, since in selecting "slows" one might be choosing potentially "quicks". I am quite sure that in my records there are "slow" rats entered as "quicks", and "quicks" entered as "slows", and that there are far more instances of the latter than of the

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former. So many factors are concerned in the establishment of the score that it is improbable that an experiment such as this could reveal individuals whose scores were commonly the direct expressions of their genotypes, uninfluenced by non-genetic factors. The average score of the 683 males of the experimental group is 45.17, that of the 762 females 41.79. That of the 586 rats of Line A is 30.8 (males 28.05; females 32.11); that of the 564 rats of Line B is 56.88 (males 61.87; female 51.90). These differences have been examined statistically; the scores of Line A rats are significantly lower on the average than are those of Line B rats: within Line A there is no significant difference due to sex, within Line B are less than 20 to 1 that the males have a higher score than the females.

Accepting the suggestion that only two main classes of rats are concerned, "quick" and "slow", and, since the median of the controls is 24, that quick rats are those with scores of ≤ 23 , whilst slows are those with ≥ 24 , it becomes possible to summarise the records of a sufficiently large group of the experimental stock. It will be noticed that there is

TABLE VI

Parents	...	Quick \times Quick		Quick \times Slow		Slow \times Slow		Totals	
Offspring	...	Quick	Slow	Quick	Slow	Quick	Slow	Quick	Slow
Line A		271	149	15	17	12	55	298	221
" B		35	64	6	14	77	211	118	289
" C		32	38	8	21	6	25	46	84
" G		—	—	9	16	24	79	33	95
" K		3	4	—	—	6	—	9	4
Total		341	255	38	68	125	370	504	693
Percentage		57	43	36	64	25	75	42	58
Pairs of sibs:									
Quick		3926	2275	78	144	400	852	4404	3271
Slow		2275	2138	144	254	852	2518	3271	4910
Average no. of offspring per mating		588 55	=11	106 17	=6	495 63	=8	1189 135	=9
Standard deviation		9		2		4½		7	

a preponderance of quick \times quick matings in Line A, and of slow \times slow matings in Line B. Quick \times slow matings are rare; they were made only when it was not possible to mate quick with quick or slow with slow. For this reason the two lines have come to be different in respect of the proportions of quicks and slows in the three types of litter. The figures seem to suggest that quickness and fertility are connected in some way. I think they are misleading. Obviously quick by quick matings can produce more offspring during the course of an experiment limited in

time than can slow by slow matings of the same generations if mating is deferred until training is completed. The quicks can have produced a litter and this can be half-way through its training before the slows of the same generation have been mated. For this reason the quicks have more entries in the records than have the slows. But it is the case that I have never had any offspring out of a rat with a score of 200+. I have no satisfying reason to offer for this.

The parent-parent correlation is 0.84, the parent-offspring 0.3, and the sib-sib 0.17. Undoubtedly the system of mating quick with quick and slow with slow is responsible for the first, and this must affect the other two coefficients. Nevertheless, the parent-offspring figure is such as to make it certain that genetic factors are largely concerned in determining the score that a given individual shall make. Since there is no difference in respect of the score between controls and experimentals and between the later and earlier generations of the experimentals, it follows that there is no need to postulate that any quality has been induced as a consequence of training, for the results obtained are interpretable in simple genetic terms. If the average score per rat of one generation differs from that of a preceding generation, then this difference merely means that in the two generations there are different proportions of genetically quicks and slows.

The hypothetical correlations occurring in a system of random mating and estimated by using the percentages instead of the actual figures are parent-parent 0, parent-offspring 0.17. On the basis of assortative mating, and neglecting all quick \times slow matings, and using not the percentages but the actual figures, the parent-parent correlation is 1, parent-offspring 0.4, and the sib-sib 0.18. If the 9 class classification is used instead of the 2 class, the parent-parent correlation is 0.8, and the parent-offspring 0.3. Since these are the same whether 2 or 9 classes are considered, it can be assumed that the sib-sib correlation of the 9 class would not be far removed from 0.1.

I do not propose to carry this investigation further: I willingly bequeath its interest and labour to Agar. But I propose to carry the study of photophobia and of handedness further, and reserve a discussion of these phenomena for a future occasion. For the present I must be content with the statement that handedness is an enduring and a stable characteristic, and that if it is inherited its mode of transmission is not simple and straightforward. Neither is that of photophobia, but it is the case that this phenomenon is encountered only in certain strains.

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X. SUMMARY

In order to be in a position to examine the conclusions which McDougall has reached, 18 generations of rats have been trained. The figures provided by 1445 experimentals and 1014 controls are compared with those which McDougall derived from 21 generations of rats of the same origin.

Criticism is levelled at the methods adopted by McDougall for presenting his figures, and especially at his lack of adequate control.

In the case of my rats the average number of errors per rat made by individuals of the tank-trained stock has not decreased with the passing of the generations, and there is no difference whatsoever between the scores of the experimental and control stocks. I have encountered no evidence which would suggest that rats of the trained and control stocks respectively can be distinguished one from the other by differences in behaviour. This being so I cannot accept the results which McDougall obtained as being in themselves strong enough to carry the interpretation that he has placed upon them.

Analysis of my own pedigrees shows definitely that genetic factors are heavily concerned in the establishment of the scores. Two main classes of rats are involved, quick and slow, and in a general way quickness behaves as a dominant, slowness as a recessive. The parent-offspring correlation is 0.3. A "quick" strain has been developed as the result of consistent favourable selection. To "fix" a slow strain has proved to be a much more difficult task. The reasons for this are discussed.

I submit that there is no need to postulate, in explanation of the fact that the average scores of the earlier and later generations of McDougall's rats differ, that some new quality has been acquired and is being transmitted, for the average score of a generation is determined by the proportion of quick and slow rats within it, and these proportions can, within limits, be prearranged.

Among my rats there is a great excess of those which tend to leave the tank habitually by one route during the first phase of their training when the light is constant and equal on both sides of the tank and when the platforms are not alive. A considerable number of rats reacted to light as light in the second phase of their training when the light was alternating but the platforms were not alive. Actually 29 experimental and 10 control rats "learnt" without receiving a single shock.

The relation of handedness and photophobia to the score is discussed.

I do not propose to carry the main study further. The search for the genetic basis of handedness and of photophobia is being continued.

TABLE VII

Controls

I	0, 1, 1, 2, 3, 4, 4, 4, 4, 5, 5, 5, 5, 5, 6, 6, 6, 7, 7, 7, 8, 8, 8, 8, 8, 8, 10, 10, 10, 10, 10, 11, 11, 11, 11, 11, 12, 13, 14, 14, 14, 15, 15, 15, 15, 16, 16, 16, 16, 16, 16, 17, 18, 18, 19, 19, 19, 19, 20, 20, 20, 20, 20, 21, 21, 22, 22, 22, 22, 23, 24, 24, 25, 25, 26, 26, 26, 27, 27, 28, 29, 29, 29, 30, 31, 31, 32, 37, 38, 38, 39, 40, 45, 46, 46, 56, 81
II	0, 1, 2, 2, 4, 6, 7, 8, 8, 9, 10, 10, 11, 11, 12, 13, 13, 14, 15, 16, 16, 17, 17, 18, 19, 19, 19, 20, 22, 24, 26, 31, 33, 34, 38, 41, 45, 52, 63, 70, 89, 92, 113, 115, 117, 122, 142, 174, 183, 194
III	0, 4, 7, 11, 11, 14, 17, 18, 23, 24, 25, 25, 25, 26, 26, 28, 29, 29, 29, 33, 33, 34, 37, 37, 38, 40, 41, 44, 47, 47, 48, 52, 54, 58, 59, 73, 75, 77, 94, 102, 103, 107, 111, 116, 128, 169, 174, 182, 187, 291
IV	1, 1, 1, 2, 2, 3, 3, 3, 3, 3, 4, 5, 7, 7, 7, 10, 11, 12, 12, 14, 14, 16, 18, 19, 19, 20, 22, 22, 23, 24, 24, 29, 29, 33, 34, 36, 51, 60, 64, 85, 85, 89, 93, 101, 102, 106, 109, 113, 179
V	5, 7, 7, 8, 9, 9, 9, 13, 14, 16, 16, 18, 21, 23, 23, 25, 27, 28, 29, 29, 29, 30, 34, 36, 36, 38, 39, 42, 47, 48, 52, 55, 57, 57, 59, 64, 67, 73, 76, 78, 85, 92, 97, 101, 105, 107, 111, 129, 275
VI	4, 6, 7, 7, 9, 9, 9, 11, 11, 13, 15, 17, 17, 19, 20, 21, 23, 23, 24, 24, 26, 28, 31, 33, 35, 42, 43, 43, 52, 54, 56, 58, 61, 61, 63, 65, 67, 72, 84, 88, 97, 98, 99, 103, 105, 107, 110, 114, 115, 123
VII	8, 10, 12, 15, 16, 18, 19, 19, 20, 21, 23, 24, 27, 27, 29, 32, 36, 39, 39, 39, 40, 41, 41, 44, 46, 49, 52, 54, 56, 57, 58, 64, 66, 66, 66, 72, 81, 87, 88, 89, 94, 97, 103, 105, 111, 132, 139, 152, 173, 194
VIII	2, 4, 4, 5, 7, 7, 7, 8, 8, 9, 9, 9, 10, 10, 10, 11, 11, 12, 13, 14, 14, 15, 15, 16, 16, 16, 17, 17, 18, 19, 20, 20, 20, 21, 22, 24, 24, 26, 26, 29, 31, 33, 35, 39, 42, 45, 57, 58, 86, 94
IX	2, 4, 4, 4, 6, 6, 6, 7, 7, 7, 7, 8, 9, 10, 11, 11, 11, 13, 14, 14, 15, 15, 15, 16, 16, 17, 17, 18, 18, 18, 21, 21, 22, 23, 31, 35, 37, 44, 48, 56, 71, 77, 89, 102, 105, 113, 115, 120, 162
X	4, 7, 11, 14, 15, 21, 24, 27, 32, 34, 35, 35, 37, 38, 41, 43, 44, 47, 48, 49, 53, 54, 57, 57, 58, 62, 63, 65, 67, 67, 71, 71, 72, 73, 74, 76, 78, 84, 87, 89, 96, 104, 113, 125, 133, 152, 174, 181, 193, 201
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XII	2, 3, 3, 4, 5, 5, 6, 6, 6, 7, 7, 8, 8, 8, 9, 9, 10, 10, 10, 10, 11, 11, 11, 12, 13, 15, 17, 17, 19, 19, 19, 22, 24, 24, 26, 26, 28, 31, 34, 36, 39, 39, 42, 44, 47, 49, 61, 93, 95, 101
XIII	0, 0, 1, 2, 2, 2, 3, 3, 4, 4, 4, 5, 5, 5, 6, 7, 7, 7, 9, 10, 10, 12, 14, 15, 17, 21, 24, 26, 26, 29, 31, 33, 36, 38, 47, 51, 57, 62, 64, 71, 74, 83, 91, 92, 97, 99, 99, 104, 116, 121
XIV	4, 7, 8, 9, 9, 11, 12, 14, 14, 14, 15, 15, 15, 15, 16, 17, 17, 19, 19, 23, 23, 25, 25, 26, 26, 27, 27, 29, 29, 31, 32, 34, 47, 49, 52, 57, 59, 67, 69, 81, 92, 97, 99, 107, 113, 124, 148, 205, 279, 291
XV	1, 2, 4, 4, 5, 6, 7, 7, 7, 9, 9, 12, 12, 14, 15, 16, 16, 18, 21, 23, 27, 29, 32, 33, 35, 36, 38, 39, 43, 47, 49, 57, 64, 83, 89, 97, 99, 104, 117, 134, 162, 167, 181, 193, 197, 204, 231, 259, 271, 298
XVI	2, 3, 4, 4, 7, 7, 9, 11, 12, 16, 17, 19, 19, 19, 20, 21, 25, 27, 27, 28, 28, 29, 29, 29, 31, 33, 34, 34, 36, 41, 45, 46, 48, 53, 56, 58, 67, 79, 82, 88, 89, 89, 93, 98, 103, 107, 114, 137, 152
XVII	2, 5, 7, 7, 8, 8, 10, 11, 11, 11, 12, 12, 12, 13, 15, 18, 19, 19, 23, 25, 28, 29, 31, 33, 37, 44, 46, 49, 50, 51, 54, 56, 61, 63, 66, 69, 72, 76, 79, 83, 85, 87, 89, 94, 97, 104, 111, 131, 163, 216
XVIII	0, 0, 0, 0, 1, 1, 1, 1, 2, 2, 2, 2, 2, 3, 3, 3, 3, 4, 4, 4, 4, 4, 5, 5, 5, 5, 5, 5, 5, 5, 6, 6, 6, 7, 7, 7, 7, 8, 8, 8, 8, 9, 9, 10, 11, 11, 11, 11, 12, 12, 12, 12, 12, 12, 14, 14, 14, 14, 15, 16, 17, 18, 19, 19, 20, 20, 20, 21, 21, 22, 23, 24, 25, 26, 26, 26, 26, 28, 30, 31, 31, 32, 37, 39, 40, 42, 50, 58, 59, 59, 61, 64, 67, 68, 76, 79, 83, 84, 89, 90, 92, 93, 97, 100, 113, 116, 133, 144

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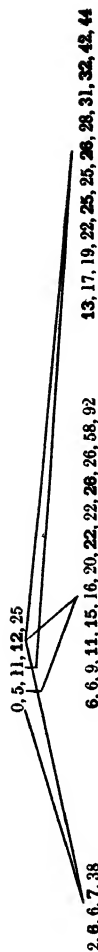
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Line C

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0, 16, 34, 41, 48, 66

57, 89, 97

7, 65, 291

Black type = a male.

ON THE GENETICAL CONSTITUTION OF *DROSOPHILA PSEUDO-OBSCURA*, RACE A

By H. P. DONALD

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(With Two Text-figures)

SOME seventeen years ago the researches of Metz on the chromosome groups of the various species of *Drosophila* induced Lancefield (1922) to undertake the study of the genetic constitution of *D. obscura* in the hope of securing evidence of the exact relationship of this species to others for which genetic data were available. Since the publication of his extensive work on sex-linked mutants there appears to have been little study of the homology of mutant loci in this species (now *D. pseudo-obscura* Frol.). More recently the recognition of the value of *D. pseudo-obscura* as a means for investigating the process of species formation has led to more intensive study and the accumulation of further genetic data. At the same time, the development by Muller of the X-ray technique for inducing an increased rate of mutation and of chromosome rearrangement, and the demonstration by Painter of the possibilities of the salivary gland method of chromosome study, have thrown into prominence the various kinds of change in the constitution of chromosomes which may have played a part in the evolution of species. It has seemed worth while, therefore, to sum up our present knowledge of the mutant forms of *D. pseudo-obscura*, Race A, in order to see what light may be shed on the relationship of this to other species and on the processes which led to their differentiation. With this end in view, maps have been prepared which summarise the linkage data at present available. Using these maps as a basis for study, the mutant forms have been compared with possible homologues in other species and the suggestions arising therefrom outlined.

I. THE LINKAGE MAPS

Apart from the linkage map of the sex-linked characters which has been published previously by Lancefield (1922), and the summary of the then known mutants in all linkage groups given by Morgan, Bridges and Sturtevant (1925), there have been no published maps in which to in-

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corporate new data. With the exception, therefore, of the sex-chromosome, the map for which has been based on Lancefield's and includes all his mutants, the chromosomes are represented here by completely new maps (Fig. 1). Although none of the mutants which Lancefield located in the autosomes have been used, it appears fairly certain that the linkage groups as numbered here correspond with his, partly because there have been reappearances of some of his mutants, and partly because some of them appear to have been available elsewhere.

The maps do not represent a summary of accurate linkage data. At present they serve merely as a groundwork for further more detailed investigation of the linkage properties of these chromosomes, and to indicate the kind and position of the landmarks which are available for the study of chromosome constitution. It has not been considered advisable at this stage to adopt the methods available for the construction of accurate linkage maps. The simple procedure of using the cross-over percentages, calculated from the primary data given in Table I, as map distances has been followed. Most of the data for each distance is derived from one linkage experiment, and represents the frequency of single cross-overs between the loci indicated in column 4 of the table. Lancefield's data of 1922 are not included because the revised map given by Morgan, Bridges and Sturtevant (1925) is different in some respects and is not accompanied by the figures on which it is based.

The map for the *X*-chromosome from *Pointed* to *compressed* is practically the same as Lancefield's except that new loci are shown and that *singed* is placed to the left instead of to the right of *vermilion*. Four experiments agreed fairly closely in giving about 42 per cent. of recombination between *dusky* (74) and *sepia* (Donald, 1936), so that 116 has been taken as the locus of *sepia*, and as the basis of reference for mutants to the right of it. The occurrence of three new mutants between Lancefield's *compressed* and *ascute* has permitted shorter distances to be measured and has thus had the effect of shifting *short* and the mutants near it farther to the right than appears in his map.

It has not been possible to find a satisfactory starting-point for the second linkage group. *Smoky* has been placed provisionally at 0.0 on the grounds that Tan's (1935) figure of the inversion found by Sturtevant to reduce crossing-over between *Bare* and *Smoky* indicates that one of these two mutants has a locus somewhere near the end of the chromosome. Since *Stubble* and *glass* occur at considerable distances upon either side of *Bare*, it seems likely that *Smoky* is the one nearest the end.

Another unsatisfactory feature of the map for the second chromosome

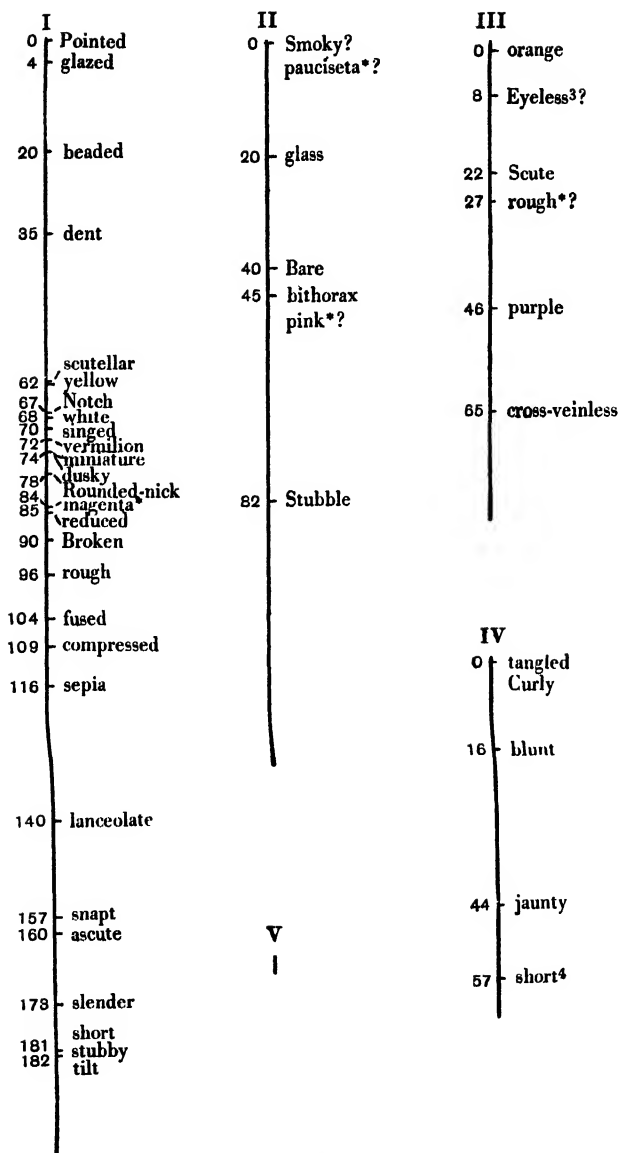


Fig. 1. Current linkage maps for *D. pseudo-obscura*. The position of mutants marked (?) is still doubtful. Mutants received from Dr Dobzhansky and not located with reference to mutants used in constructing these maps are marked with an asterisk.

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is that the loci for *Stubble*, *glass* and *bithorax* have been determined with relation to *Bare* and not to *Smoky*, and consequently each may lie on the opposite side of *Bare* to that shown. There are no published accounts of crossing-over between *Smoky* and *Bare*, but there are indications that it is about 40 per cent. The amount of information available about this linkage group is shown in Table I and is clearly inadequate for constructing a map containing all the known mutants. It may be mentioned that the mutant *aristipedia* has been found to belong here also.

TABLE I

Summary of recent linkage experiments carried out on D. pseudo-obscura. Flies were cultured at 23.5 ± 0.5° C. and counted over a period of about 10 days

Locus	Mutant	Symbol	Loci tested	Cross-overs	Total	% crossing-over	Cross for order
X-chromosome							
35	dent	<i>de</i>	<i>y-de</i>	858	3183	27.0	<i>b</i> × <i>y</i> <i>de</i>
70	singed ²	<i>sn</i> ²	<i>v-sn</i>	7	468	1.5	<i>m</i> ² × <i>y</i> <i>v</i> <i>sn</i> ²
72	vermillion	<i>v</i>	<i>y-v</i>	639	6551	9.8	<i>m</i> ² × <i>y</i> <i>v</i> <i>sn</i> ²
74	miniature ²	<i>m</i> ²	<i>v-m</i> ²	21	1212	1.7	<i>m</i> ² × <i>y</i> <i>v</i> <i>dy</i> <i>se</i>
74	dusky	<i>dy</i>	<i>v-dy</i>	21	1212	1.7	<i>m</i> ² × <i>y</i> <i>v</i> <i>dy</i> <i>se</i>
78	Rounded-nick	<i>Rn</i>	<i>v-Rn</i>	117	2006	5.8	<i>y</i> × <i>v</i> <i>Rn</i>
116	sepia	<i>se</i>	<i>dy-se</i>	1579	3778	41.8	+ × <i>b</i> <i>dy</i> <i>se</i> <i>sp</i>
140	lanceolate	<i>ll</i>	<i>se-ll</i>	956	3938	24.3	<i>ll</i> × <i>b</i> <i>dy</i> <i>se</i> <i>sp</i>
157	snapt	<i>sp</i>	<i>ll-sp</i>	267	1534	17.4	<i>sp</i> × <i>se</i> <i>ll</i>
181	short	<i>s</i>	<i>sp-s</i>	131	541	24.2	<i>s</i> × <i>se</i> <i>ll</i> <i>sp</i>
182	tilt	<i>tt</i>	<i>ll-tt</i>	28	67	41.8	<i>tt</i> × <i>se</i> <i>ll</i>
2nd chromosome							
20	glass	<i>gl</i>	<i>bx-gl</i>	672	2678	25.1	<i>Sb</i> × <i>gl</i> <i>bx</i>
40	Bare	<i>Ba</i>	—	—	—	—	—
45	bithorax	<i>bx</i>	<i>Ba-bx</i>	34	705	4.8	<i>Ba</i> × <i>gl</i> <i>bx</i>
82	Stubble	<i>Sb</i>	<i>bx-Sb</i>	724	1973	36.7	<i>Sb</i> × <i>gl</i> <i>bx</i>
3rd chromosome							
0	orange	<i>or</i>	—	—	—	—	—
8?	Eyeless ³	<i>Ey</i> ³	<i>pr-Ey</i> ³	98	257	38.1	<i>pr</i> × <i>Ey</i> ³
22	Scute	<i>Sc</i>	<i>or-Sc</i>	826	3828	21.6	<i>Sc</i> × <i>or</i> <i>pr</i>
46	purple	<i>pr</i>	<i>Sc-pr</i>	906	3828	23.7	<i>Sc</i> × <i>or</i> <i>pr</i>
65	cross-veinless	<i>cv</i>	<i>pr-cv</i>	662	3528	18.9	<i>Sc</i> × <i>or</i> <i>pr</i> <i>cv</i>
4th chromosome							
0	tangled	<i>tg</i>	—	—	—	—	—
0	Curly	<i>Cy</i>	<i>tg-Cy</i>	0	144	0.0	<i>tg</i> × <i>Cy</i>
16?	blunt	<i>bl</i>	<i>tg-bl</i>	55	337	16.3	<i>tg</i> × <i>bl</i>
44?	jaunty	<i>j</i>	<i>tg-j</i>	654	1490	43.9	<i>tg</i> × <i>j</i> <i>s</i> ₄
57?	short ₄	<i>s</i> ₄	<i>j-s</i> ₄	445	3477	12.8	<i>tg</i> × <i>j</i> <i>s</i> ₄

Following Lancefield, the locus of *orange* on the third chromosome has been placed at 0.0, but, judging from the position of the *Scute* inversion (Tan, 1935) in that chromosome and the fact that there is not much crossing-over between *orange* and the inversion, *orange* is probably

some distance from the end of the chromosome. No three-point experiment has been made with *Eyeless*, so that its position is doubtful.

In the fourth linkage group the mutant *tangled* has been given the locus 0.0, not because there is any indication of its whereabouts on the chromosome, but merely because it is at present at one end of the group and is used for fixing the position of other members of the group. On the basis of a small experiment *Curly* is placed also at the locus 0. Although it is quite possible for two different loci to show no crossing-over, especially with small numbers, there is a suspicion that these two mutants may be allelomorphic because *Cy/tg* flies show *Curly* and slight tangling at the end of L_2 and L_3 . *Curly* is expected to show but not *tangled* which is a good recessive. As an alternative to this unusual type of allelomorphism, it may be supposed that *Curly* causes *tangled* to become partially dominant in somewhat the same way as *Abrupt-X* causes *scute* to become partially dominant in *D. melanogaster* (Nazerenko, 1930). Although the *jaunty-short*₄ distance is known fairly accurately, they are both so far away from *tangled* that it has not been possible to determine which is the nearer. Doubtless the use of the intermediate mutant *blunt* will enable this matter to be settled shortly.

Through the kindness of Dr Dobzhansky, we have received a number of mutants from the California Institute of Technology, and these have been included in the maps at the provisional loci assigned at Pasadena. With the exception of *Bare* and *Curly* their relation to our mutants has not yet been determined, and they have therefore been identified on the maps with an asterisk.

The total number of mutants recorded in these maps is 45, but this number will doubtless be augmented shortly by a considerable number of mutants in hand here and elsewhere. Of these 45 mutants, 10 are dominants, and of these 10, 6 are viable when homozygous, and 4 lethal when homozygous (Notch, Broken, Curly, Eyeless). The mutant *Bare* is also recorded as lethal when homozygous, but in stocks recently obtained all flies are uniformly *Bare*. This cannot be due to a balanced lethal system as F_1 flies from an out-cross are all *Bare*. Twenty-seven of these mutants are sex-linked (4 dominant), and 18 autosomal (6 dominant). It will be observed that the sex-chromosome accommodates an unusually long linkage group: even now with several large gaps of uncertain length it appears to be at least 182 units of crossing-over long, and there is little doubt that when all double crossing-over can be detected and possible mutants beyond the farthest now known discovered, it will prove to be nearly 200 units long. As will be indicated later, it

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seems likely that this is due partly to a high rate of crossing-over per unit of chromosome length as well as to the actual length of the chromosome.

There is still no well-established mutant locus for the small fifth linkage group, but for each of the remaining autosomal groups there are at least four well distributed workable mutants including a dominant, so that the localisation of further mutants should be fairly rapid.

II. COMPARISON OF MUTANTS IN *D. PSEUDO-OBSCURA* WITH SIMILAR ONES IN *D. MELANOGASTER*

A. *Sex-linked mutants*

(a) *Pointed (P) and beaded (b)*. Lancefield (1922) and Koller (1932) described in some detail the mutant *Pointed* of which a second occurrence and two reversions to wild-type have been observed. These authors discussed its possible homology with the *Beaded* of *D. melanogaster* (3-93·8), which, under the influence of selection, produced a wing very like that of *Pointed*. *Beaded* is unlike *Pointed* in being lethal when homozygous and in causing a much smaller reduction of the wing when obtained from outcrosses (Bridges and Morgan, 1923) than *Pointed*. While these differences in themselves do not rule out the possibility of homology, they make it a rather doubtful one, especially as a possibly better case could be made out for the correspondence of *Pointed* with *Beadex* of *melanogaster* (1-59·4). The importance attaching to the homology of *Pointed* lies in the necessity of explaining the relationships of that portion of the X-chromosome of *D. pseudo-obscura* which lies to the left of the locus for *yellow*. This question is narrowed somewhat by a consideration of the mutant *beaded* which shows a very close resemblance to the *cut* of *D. melanogaster* (1-20·0). The wings of some types of *beaded* are very similar to the figures of *cut* given by Morgan, Bridges and Sturtevant (1925, p. 35). Koller (1932) figures another type. In both species the mutants are sex-linked recessives with a considerable range of genetic variability and a high rate of mutation. Numerous *cut* allelomorphs have been recorded, and *beaded*, which is probably the most frequently recurring mutation in *D. pseudo-obscura*, has put in at least ten fresh appearances in this laboratory. Unlike *cut*, *beaded* causes no observable effect on the eye shape or on the arista, and the flattening of the distal antennal joint seen in *beaded* flies is characteristic of wild-type *D. pseudo-obscura*. If *beaded* and *cut* be now considered to correspond, the speculation might be made that part at least of the *Pointed*-

yellow region of the *D. pseudo-obscura* X-chromosome (with *cut* (*beaded*) transferred intrachromosomally) had the same origin as the *Beaded* region of the X-chromosome of *D. melanogaster*, and that if *bobbed* should occur in *D. pseudo-obscura* there is a possibility of its being found between *Pointed* and *yellow* (see Postscript).

(b) *Yellow* (*y*) and *scutellar* (*sc*). As a rule, the comparison of mutants causing bristle defects is hardly worth while on account of the large number of such mutants, but the proximity of *scutellar* to *yellow* as noted by Lancefield (1922) creates a high degree of probability that these mutants are both represented in *D. melanogaster* and other species in which *yellow* and *scute* occur together (Morgan, Bridges and Sturtevant, 1925, p. 207). The appearance of *yellow* on at least two occasions, and of a *yellow* allelomorph called *cuprous* accords well enough with the observed mutability of the *yellow* locus (1-0-0) in *D. melanogaster*. Both mutants are quite recessive and of good viability. The locus of *yellow* in the X-chromosome of *D. pseudo-obscura* is, however, not terminal as in *D. melanogaster*, but sub-median, and some 60 crossing-over units from *Pointed*. According to Lancefield's data, *scutellar* is on the left side of *yellow*, whereas in *D. melanogaster* it is on the right.

(c) *White* (*w*). Three mutations at the *white* locus, including an *eosin*, were discovered by Lancefield (1922). Since then at least three other spontaneously occurring *whites* have been recorded, and of these *w*⁵ and *w*⁶ are certainly not female-sterile as were *w*¹ and *w*². In respect of appearance, allelomorphic series, mutability, sexual dimorphism of *eosin* and inhibiting effect on *vermilion*, *white* shows a very good correspondence with the *white* of *D. melanogaster* (1 1·5).

(d) *Notch* (*N*). A *Notch* character located close to *white*, and acting as a recessive lethal has been compared to the *Notches* of other species by Lancefield (1922). Though it has not been cytologically demonstrated that his *Notches* were caused by deficiencies there can be little doubt that there is a section of chromosome near *white* which is represented in various species and which has retained its tendency to become deleted.

(e) *Singed* (*sn*). Lancefield (1922) reported the occurrence of a fairly slight form of *singed* which was female-sterile, recessive, sex-linked and generally similar to the *singed* of *D. melanogaster* (1-21·0). Two *singed* allelomorphs have been found here which differ to some extent from this description. From the point of view of homology, the distinction between *singed* and *forked* is one of some importance and has been investigated in detail by Metz, Moses and Mason (1923) who could find no reliable criteria for distinguishing between them. Short descriptions of these two

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singed mutants are given, and though it seems that they are closer to the general *singed* type, it is clear that it is impossible to be sure that they are not really *forked*.

*Singed*² (*sn*²) (Crew and Lamy, unpublished) is a very slight form in which only the long bristles are affected. These are wavy or slightly curled and not much reduced in length. The shorter bristles, hairs and eggs appear to be normal. Low viability and poor separability make it inferior for linkage work.

*Singed*³ (*sn*³) (Crew and Lamy, unpublished) is an extreme type. All long bristles on the head and thorax are strongly gnarled and depressed, and the short ones slightly wavy. Short acicular branches occur on the arista, and the hairs on the third antennal segment are slightly wavy. The eggs are normal and the viability and fertility good.

*Singed*⁴ (*sn*⁴) which occurred recently appears to be intermediate between these two, but closer to *singed*³.

Since it has not been possible to test Lancefield's *singed* with any of these, it is only an assumption that they belong to the same locus. Lancefield's data indicated that *singed* lay to the right of *vermilion*, but recently evidence has been obtained (Crew and Lamy, unpublished) that it lies to the left between *vermilion* and *white*. This makes the order of the loci for *y*, *w*, *sn*, *v*, the same for both *D. melanogaster* and *D. pseudo-obscura*, although the linkage values involved do not agree.

(f) *Vermilion* (*v*). A sex-linked eye colour (Crew and Lamy, 1934) very similar to the mutant *vermilion* in *D. melanogaster* (1-33-0) was found by Lancefield (1922) and two other spontaneous occurrences have been noted here. Because of its similarity to *vermilion*, its position in the linkage group, and its interaction with *white*, there is a presumption in favour of homology. On the other hand, mutations in a variety of sex-linked and autosomal loci produce something like this colour in *D. melanogaster*, and give the same interaction with *white*. If, further, the gene producing *vermilion* does so by preventing the production of brown pigment, then it would have to eliminate more brown pigment in *D. pseudo-obscura* in order to bring about the same effect. This, of course, is not a real difficulty if the gene for *vermilion* has no quantitative connection with the absence of brown pigment. The objection to homology raised by Lancefield (1922) on account of the yellow colour of the *eosin-vermilion* compound in *D. pseudo-obscura* cannot be considered very weighty as there may be just this degree of difference in the production of yellow pigment in the wild-type eyes of the two species.

(g) *Miniature* (*m*) and *dusky* (*dy*). As with *scute* and *yellow*, the

appearance of these two characters with closely linked loci in several species provides good evidence of their homology. Lancefield did not intend to signify that his *miniature* found in *D. pseudo-obscura* was the same as that in *D. melanogaster* (1-36.1), but the correspondence of the mutants in appearance, viability, variability and mutability is good. They agree, also, in a tendency to hold the wings extended. As shown previously (Donald, 1936) *dusky* also shows a close similarity to the *dusky* (1-36.2) of *D. melanogaster*. Unfortunately, the exact relations of *vermilion*, *miniature* and *dusky* to each other in *D. pseudo-obscura* have not yet been determined, so that it is impossible to say whether they lie in the same order as in *D. melanogaster*. *Miniature* and *dusky* are so closely linked in *D. pseudo-obscura* that so far there has been no crossing-over between them, and it is therefore impossible to decide which is the nearer to *vermilion*.

(h) *Magenta* (*mg*). This mutant has been described fairly fully by Sturtevant and Dobzhansky (*D.I.S.* 1, 41) and compared with the *magenta* (1-73.7) of *D. virilis* which to Metz, Moses and Mason (1923) suggested the *garnet* (1-44.4) of *melanogaster*, and the *carmine* (*garnet*?) of *simulans*. As indicated under *vermilion*, the probability of error in deciding on the homology of eye colours is high, and consequently the resemblance of these two mutants and the similarity of the position of their loci with reference to the foregoing sex-linked characters, although suggestive, can only be regarded as of doubtful significance.

(i) *Sepia* (*se*). This mutant which was named *sepia* on account of its resemblance to the *sepia* of *melanogaster* (Crew and Lamy, 1934) comes into that category of eye mutations which are characterised by an extra and post-pupal development of brown pigment. Several eye mutants in *D. melanogaster*, therefore, could possibly be corresponding, but the degree of darkening after emergence points to *sepia* (3-26.0) as the most likely.

(j) *Lanceolate* (*ll*). Since no figures of the mutants called *lanceolate* or *narrow* etc. in other species have been seen, too great significance should not be attached to the use of the name *lanceolate* here. Nevertheless it is a fairly distinct type of mutation, and the choice of possibilities is not a very wide one. In Fig. 2 it is tentatively made to correspond with the *lanceolate* (2-106.7) of *D. melanogaster*.

(k) *Short* (*s*), *snap* (*sp*) and *tilt* (*tt*). In spite of the inherent difficulties in seeking corresponding mutants for these vein abnormalities the temptation to trace the affinities of the right arm of the *X*-chromosome of *D. pseudo-obscura* has led to the present attempt. *Short*, which

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is very similar to *short*₄, may be compared with the abrupt ($2.42 \pm$) of *D. melanogaster*. *Short*₄ may equally well be compared with the latter, and whether either, both or neither are homologous is a matter still to be made clear.

As pointed out by Crew and Lamy (1934), *snapt* seems to match the *radius incompletus* (3.46.0) of Borissenko (1930) fairly well. Both are recessive and of good viability, and appear to affect only the *L*₂ even in the most extreme condition.

Although the *tilt* of *D. pseudo-obscura* is more like the *tilt* (3.40.2) of *D. melanogaster* than any other mutant described, the agreement is not complete. Gaps in the third longitudinal vein associated with an upward tilt to the end of the wing occur in both mutants, but the *tilt* flies of *D. pseudo-obscura* hold the wings in the normal position and have *L*₃ usually short instead of broken. Since some *tilt* stocks show *L*₂ and *L*₄ short as well as *L*₃, an effect which may also be produced by introducing lanceolate, it is clear that the expression of *tilt* is easily modified. This may account for its divergence from the *tilt* of *D. melanogaster*.

(l) *Rough* (*ro*) and *ascute* (*as*). On the basis of the descriptions given by Lancefield (1922) these two mutants have been compared with the mutants *roughoid* (3.0.0) and *ascute* (3.43.5) of *D. melanogaster*. The probability that the *ascutes* are the same seems quite high, but the case for the two *roughs* is weak.

B. Autosomal mutants

(a) *Bithorax* (*bx*), *glass* (*gl*), *Stubble* (*Sb*). This group of mutants corresponds very well with those of the same name in *D. melanogaster*. At present it is immaterial whether *bithorax* is assumed to be homologous with *bithorax* (3.58.7) or *bithoraxoid* (3.59.5) of *D. melanogaster* since they are so close together, but the fact that there can be some doubt about the homology of such a distinctive character as this illustrates very well the danger of assuming correspondence without a direct mating test.

Glass seems to correspond well with its counterpart in *D. melanogaster* (3.63). From the descriptions and figures (Bridges and Morgan, 1923) the latter appears to have a colourless rim which is part of the eye and which is caused by limitation of pigment to the central ommatidia. There is also a distinctive colourless rim in the *glass* of *D. pseudo-obscura*, but here it is due to the reduction in size of the eye which results in the formation of a bright chitinous ring round the eye. The only real difference from the *glass* of *D. melanogaster* appears to be that the pigment granules (which are very coarse in both mutants) are distributed

in a thin layer just below the facets and extend to the edges of the eye instead of being limited to the central part as in *D. melanogaster*.

As noted by Crew and Lamy (1935), there is a strong resemblance between the *Stubble* of *D. pseudo-obscura* and the *Stubble* (3-58.2) allelomorphs of *D. melanogaster*. The measurements made by Dobzhansky (1929) on the legs, wings and bristles of the latter demonstrated the manifold effects of the *Stubble* genes, and it is clear that the same organs are affected in the same way by the *Stubble* gene of *D. pseudo-obscura*.

(b) *Bare* (*Ba*). There seems to be some similarity between *Bare* and the *Hairless* (3-69.5) of *D. melanogaster* which also eliminates some of the microchaetae on head and thorax. Homozygous *Bare* flies are very like the *H/H*/+ flies of Gowen (1933), but heterozygous *Bare* exerts a less selective action on the bristles than heterozygous *Hairless*, and has no associated vein abnormality. The attraction of this comparison, as will be seen later, lies in its corroborative value.

(c) *Aristipedia* (*ar*). An example of hereditary homoösis (*bithorax*) has been known for some time in *D. pseudo-obscura*, and recently another has been found coming into this category, the significance of which has been shown by Bridges and Dobzhansky (1932). Mutations causing a transformation of the arista into leg-like organs have already been described (Sturtevant, 1929; Balkaschina, 1929) and appear to be much the same as the one here reported. As it was first seen in large numbers in a stock of *pr⁴tg* which is quite vigorous, it seems unlikely that the low viability investigated by Nikoro (1931) characterises the mutant in *D. pseudo-obscura* or else it would hardly have established itself in such a stock. Whilst the description by Sturtevant (1929) of the *aristipedia* of *D. simulans* applies for the most part, it is unsuitable in some respects. There is, for instance, considerable variation in the expression of the character which tends to overlap the wild-type, especially under crowded culture conditions, or at a temperature higher than that of a cool stock room. Further, the legs and bristles are normal, and the third antennal segment retains its usual shape. The extent of sterility in the females is not known. Flies with a good expression of the character show well the reduction of the branches of the arista, the thickening and segmentation of the stalk of the arista, and the two tarsal claws. A feature not mentioned by Sturtevant is the occasional expansion of the tip into a spatulate form. The interest of this mutant here lies in the fact that a prediction that its locus would be found to lie in the second linkage group has been borne out by experiment. This is not very remarkable in itself since *aristipedia*, being probably autosomal from its manner of

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discovery, must belong to one of four chromosomes. At the same time, the correctness of the prediction strengthens the supposition that the mutants *bithorax*, *Stubble* and *glass* are homologous in *D. melanogaster* and *D. pseudo-obscura* because the prediction was based on the fact that the locus for *spineless* (which is allelomorphic to *aristipedia*) is close to the loci for these mutants in *D. melanogaster*. As it has not yet been located within the linkage group, the locus of *aristipedia* does not appear on the map.

(d) *Pink* (*p*). The possibility that this *pink* is the same as the *pink* (3-48.0) of *D. melanogaster* arises as much from the position of their loci in the two species (Fig. 2) as from the superficial resemblance of the two characters.

(e) *Orange* (*or*), *Scute* (*Sc*), *purple* (*pr*). *Orange* was originally obtained from Lancefield under this name. Whether this is the same as the *scarlet* (Lancefield, 1918) mentioned in the third linkage group (Morgan, Bridges and Sturtevant, 1925, p. 201) is not known. It is indistinguishable from the sex-linked *vermilion* and resembles several autosomal mutant eye-colours in *D. melanogaster*. Comparison with *cinnabar* shows good agreement, although obviously it is impossible to seek an homologous locus for the *orange* locus on the grounds of appearance alone. This remark applies almost as well to *purple*, save that the latter has produced a series of four allelomorphs which give different interaction effects with *vermilion* (Crew and Lamy, 1934) and so suggests the *brown* (2-104.5) of *D. melanogaster*. *Brown* produces a colourless eye when combined with *vermilion* or *scarlet* (Wright, 1932), and has at least five distinguishable allelomorphs. It should be pointed out that *purple* (2-54.5, mel.) is also very similar, and its interaction with *vermilion* provided the first example of "disproportionate modification" (Bridges and Morgan, 1919).

Scute, a dominant mutation causing an elimination of some or all of the large and small bristles on head and thorax, has so far been found of little use in determining the homology of its particular section of chromosome.

(f) *Cross-veinless* (*cv*). Phenotypically, this mutant is a fairly exact copy of the *cross-veinless* (1-13.7) of *D. melanogaster*, but as shown recently (Donald, 1936) it differs in having associated with it (at 23-24°C.) a semi-lethal effect. Nevertheless, the regularity with which the cross-veins are completely missing rather favours the correspondence of these two; but there are other possibilities such as *cross-veinless-c* (3-58.3) in *D. melanogaster*.

(g) *Eyeless* (*Ey*). The occurrence of an *Eyeless*³ which is rather more extreme than the *Eyeless*² described by Donald (1936) strengthens the idea that the *Eyeless* locus is homologous with the locus of *Lobe* (2-72.0)

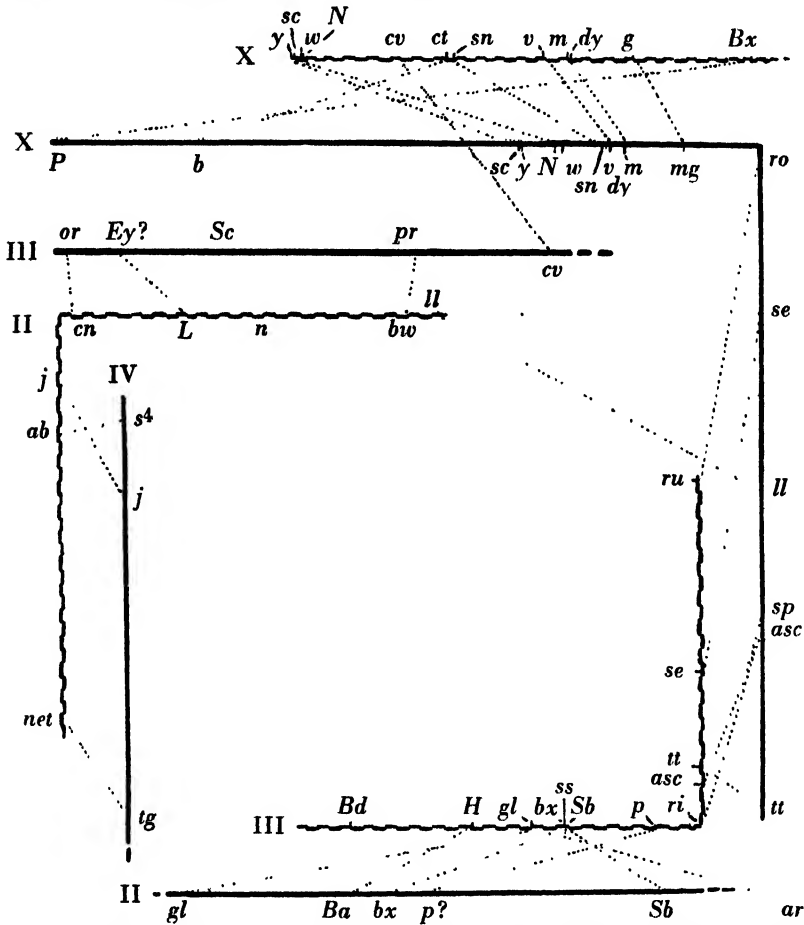


Fig. 2. Linkage maps of the major chromosomes of *D. pseudo-obscura* (straight lines) and *D. melanogaster* (wavy lines) with corresponding loci connected with dotted lines. The mutant *aristipedia* has not yet been located within the second chromosome of *D. pseudo-obscura*.

of *D. melanogaster*. Although there has been no approach to the type of *Lobe* figured by Morgan, Bridges and Sturtevant (1935) the small flat eyes with nicks in the anterior margin seem to correspond very closely to the description of *Lobe*² (Mohr, 1923) which, however, is viable when homozygous, whereas *Eyeless* possibly acts as a recessive lethal.

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(h) *Tangled* (*tg*), *short*₄ (*s*₄), *jaunty* (*j*). In describing *short*₄ and *jaunty*, Crew and Lamy (1935) drew attention to the possible homology of these loci with the loci for the *abrupt* (2-42 ±) and *jaunty* (2-48·7) of *D. melanogaster*, so nothing more need be said here about them. It was anticipated that *tangled* which has shown itself to be very susceptible of genetic modification would be of little use for purposes of homology, but it seems clear that if it is represented at all among the mutants of *D. melanogaster*, it must correspond to either *net* (2-0 ±) or *plexus* (2-100·5), with the odds in favour of the former on account of its more extreme nature. Like *net*, *tangled* is a recessive of good viability.

III. COMPARISON OF THE LOCI OF THE FOREGOING MUTANTS WITH THE LOCI OF THEIR SUGGESTED HOMOLOGUES IN *D. MELANOGASTER*

For the purposes of the discussion which follows, Fig. 2 has been drawn to show the suggested relations between various parts of the chromosome complements of *D. pseudo-obscura* and *D. melanogaster*. It shows the mutants mentioned above at their appropriate loci in the various linkage groups with the suggested homologues joined by dotted lines, and incorporates almost completely the previous diagrams of this type (Lancefield, 1922; Crew and Lamy, 1934; Koller, 1936). If there is any general validity in the conclusions expressed by this figure, there must be a close relation between the left arm of the X-chromosome and the X-chromosome of *D. melanogaster*, but there can have been little doubt of this since Lancefield published the first evidence on the point. There remains, however, the question of the portion of the chromosome to the left of *yellow*. Since one of the best cases for homology lies in the similarity of *beaded* and *cut*, it seems likely that some of this portion at any rate has the same origin as the rest of this arm. If the suggestion of Koller (1932) concerning *Pointed* is adopted, then a certain part of the chromosome in the vicinity of this locus would be homologous with the *Beaded* end of the third chromosome of *D. melanogaster*. On the other hand, it may have been derived from the source that gave rise to the locus for *Beadex* which is sex-linked in *D. melanogaster*. A striking feature of the maps for the two sex-chromosomes is the fact that at least five of the loci (*y w sn v m*) and the attachment chromomere have the same order. If sections of chromosomes containing the loci for *cut* and *cross-veinless* be deleted from the region between *yellow* and *singed* of *D. melanogaster* (or the corresponding ones inserted in *D. pseudo-obscura*) then the two chromosomes would be practically identical for the *yellow-garnet-magenta* region. Such differences as there are in cross-over values

could well enough be due to the influence of the right arm of the *X*-chromosome on the distribution of chiasmata. Concerning the homology of the right arm the evidence is not so convincing. Regarded individually, none of the mutant pairs constitute satisfactory evidence, but in the mass they are quite suggestive of a general homology of the right arm with the *sepia* arm of the third chromosome of *D. melanogaster*. The figure indicates that the evidence is better than it is, for two of the five lines are based merely on the agreement of the recorded accounts of *rough* and *roughoid*, *ascute* and *ascute*. However, these pairs accord very well with the rest, though their value is corroborative rather than intrinsic. According to recent maps (*D.I.S.* 3) the spindle-fibre attachment lies between the loci for *radius incompletus* and *ascute*; if this is so, additional inter-chromosomal exchanges would have to be invoked to account for the positions of the supposed homologues *radius incompletus* and *snapt*. Apart from this, however, there is a strong suggestion that the right arm of the *X*-chromosome of *D. pseudo-obscura* is equivalent for the most part to the *sepia* arm of the third *melanogaster* chromosome, reversed with respect to the attachment chromomeres. Since the former is obviously much longer than the latter, at least one translocation must be postulated to account for the difference in length, and various inversions to account for the difference in relative positions of the loci.

Because it has four mutants (*Sb*, *bx*, *gl*, *ar*) which may fairly safely be considered to correspond with mutants in the *Stubble* arm of the third chromosome of *D. melanogaster*, the second chromosome for the time being may be regarded as fundamentally the same. The fact that none of these four mutants could really be compared with any in *D. melanogaster* other than the ones indicated must be regarded as supporting this conclusion which was arrived at previously by Crew and Lamy (1935). For the purpose of establishing this homology, the *Hairless-Bare* comparison is not of much value; in fact, the possibility that they may correspond is more credible from the positions of their loci than from their actual similarity. Although the order of the loci *Sb bx gl* is the same for both species, the map distances between them are quite different. This presumably indicates that the process of differentiation has included inversions or deletions affecting this region. If *Bare* and *Hairless* were homologous, there would be some evidence that an inversion had actually been involved.

Suggestions concerning the affinities of the third linkage group are somewhat more speculative than those concerning any other group. The credence attaching to them is derived as much from the process of

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elimination as from direct evidence. Of the five mutants shown in Fig. 2, one, *Scute*, appears to be of no help in this connection, and another, *cross-veinless*, is best associated with the sex-linked *cross-veinless* of *D. melanogaster*. Of the remainder, two are eye-colours, and, as such, difficult to compare and open to suspicion when the comparison is made. Nevertheless, the relations indicated in the figure have something to recommend them, and may be used as a working hypothesis until further data justify or condemn them. In discussing the homology of the right arm of the X-chromosome of *D. pseudo-obscura* above, it was mentioned that if *snapt* were really homologous with the *radius incompletus* of *D. melanogaster*, the existence of the attachment chromomere between the latter and *tilt* would argue against a simple transference of all the loci mentioned, and would demand a rather special type of exchange to account for the present position of *tilt* and *snapt* relative to the attachment chromomere. This situation might therefore be used as evidence that the attachment chromomere of the third chromosome of *D. melanogaster* is actually between *radius incompletus* and *proboscipedia*. A similar type of situation would exist in connection with *purple* (*melanogaster*) if it were homologous with the *purple* of *D. pseudo-obscura*.

Little can be added to the proposal made by Crew and Lamy (1935) that the fourth linkage group of *D. pseudo-obscura* is for the most part homologous with the *Star-black* arm of the second chromosome of *D. melanogaster*. The comparison of *short*₄ and *jaunty* with *abrupt* and *jaunty* seems very reasonable and receives some support from the resemblance of *tangled* and *net*. Since the order of the loci and their relation to the spindle-fibre attachment is not yet satisfactorily established, nothing is to be gained by discussing the tentative order shown with that of the loci shown on the *melanogaster* chromosome.

IV. DISCUSSION

Many writers have shown that the main contribution of genetics to biology has been the demonstration that it is the material of the chromosomes which is mainly responsible for the phenomena of heredity and variation, and that the causes of evolution must be sought in changes taking place in that material. The methods of genetics have so far revealed the types of changes that can occur, and one of the next steps obviously is to determine the role that each of them has played in the differentiation of species. For the study of this aspect of evolutionary processes, the various species of *Drosophila* offer excellent material, handicapped though they are by the scarcity of interfertile forms. Par-

ticular interest therefore attaches to those species or subspecies which can be crossed and which offer the opportunity for making direct observations on this point, and the investigations of Sturtevant (1920, 1921) and Kerkis (1936) on *D. melanogaster* by *simulans* hybrids, and of Dobzhansky and Boche (1933), Koller (1936), Tan (1935), and Dobzhansky (1934) on the races of *D. pseudo-obscura* may be cited as examples of the valuable results obtained in this way. These results, however, can apply in the first instance only to the closely related forms from which they were obtained, and it remains to be seen to what extent the indirect but rapidly accumulating evidence from the genetic constitution of intersterile and less closely related species supports them. The present paper is an attempt to utilise the information now available for Race A of *D. pseudo-obscura* for this purpose.

From the point of view of composing a pedigree of the various species of *Drosophila*, the direct comparison of mutant forms in *D. pseudo-obscura* with those in *D. melanogaster* has no more to recommend it than any other procedure, but it has seemed more profitable in other ways to take advantage of the relatively abundant information concerning the latter than to use other less investigated species. It is not therefore to be concluded that there is a gratuitous assumption that either of these species has been derived from the other. A combined genetical and geographical study of the *Drosophilinae* will be required to elucidate their lineage.

A striking feature of the mutant forms in *D. pseudo-obscura* is the close resemblance which many of them bear to mutants in *D. melanogaster*. This resemblance applies not only to phenotypic effects but also to dominance, variability, frequency of mutation and so on. The conclusion to be drawn from this is that during the period of natural selection separating these two species a large number of loci have retained at least some of their major characteristics. This implies in turn a considerable degree of constancy in the biochemical nature of the gene and in the effects of the wild genotype upon the extreme mutant types used in experimental work.

After the work of Metz and Moses (1923) on the chromosomes of *Drosophila*, it seemed likely that these species with chromosome groups similar to *D. melanogaster* and *D. virilis* would be more closely related to each other than they would be to species like *D. pseudo-obscura* and *D. willistoni* which have larger sex-chromosomes. It is very noticeable, however, that there is a much better agreement in the sequence of corresponding loci in the sex-chromosomes of *D. melanogaster* and

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D. pseudo-obscura than there is in the sex-chromosomes of *D. melanogaster* and *D. virilis* (*D.I.S.* 2, 44). If there exists equal opportunity for the incorporation of intrachromosomal changes in the sex-chromosomes of all species, this fact would seem to show that there may not be such a wide gap between the species with long sex-chromosomes and those with short as previously supposed. As far as *D. pseudo-obscura* is concerned, there is nothing in the constitution of the autosomes as they are known at present which is at variance with this. Considering how few autosomal mutants are known in it there is a surprising amount of agreement in their grouping in the two species, from which it might be inferred that there had been no extensive intra-chromosomal exchanges since the two species diverged from the parent stock, and that the complete reshuffling of mutants to be expected in distantly related species has not occurred.

The recent work of Kerkis (1936) and Dobzhansky and Tan (1936) on the salivary gland chromosomes of hybrid larvae has shown that the failure of pairing in these chromosomes may be due not so much to inter- and intrachromosomal rearrangements as to the existence of a sort of physiological incompatibility, and it seems reasonable to suppose that this is the underlying cause of the intersterility of more widely separated forms than they worked with, because viable induced rearrangements as far-reaching as those indicated here between *D. melanogaster* and *D. pseudo-obscura* can be imagined within a species.

Dobzhansky and Tan (1936) observed in hybrids between *D. miranda* and *D. pseudo-obscura* that most of the chromosome rearrangements were intrachromosomal; that is, inversions and intrachromosomal translocations were much more frequent than interchromosomal exchanges. That this observation probably has a wider application is shown by Fig. 2 of this paper, in which comparatively few interchromosomal, but numerous intrachromosomal, translocations are indicated. As suggested by Koller (1936), this is to be expected from the sterility induced by the former type of rearrangement.

V. SUMMARY

1. A revised linkage map for the sex-chromosomes and original maps for the autosomes of *D. pseudo-obscura* are presented.
2. A comparison of the mutants of this species with similar ones in *D. melanogaster* has been made in order to obtain evidence of the kinds of difference in the genetic constitution of the two species. This evidence, although unreliable when its elements are considered separately, offers,

as a whole, support to recent work showing that in the process of differentiation of species, intrachromosomal rearrangements are more frequent than interchromosomal exchanges.

3. It indicates also that there is probably a considerable degree of homology between whole chromosomes or whole arms of chromosomes in the two species.

4. The considerable amount of agreement which was found between the comparatively few mutants of *D. pseudo-obscura* and corresponding ones in *D. melanogaster* suggests that the loci involved have retained to a large extent the same characteristics (including phenotypic effects, variability and mutability) in spite of considerable changes in position.

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POSTSCRIPT

The list of sex-linked homologues should include *glazed* (*D. pseudo-obscura*, 1-4±) which is very similar to *lozenge* of *D. melanogaster* (1-27·7). A recent mutation to female-sterile *glazed* brings the total recurrences to at least five.

THE GENETICS OF THE WENSLEYDALE BREED OF SHEEP

II. COLOUR, FERTILITY, AND INTENSITY OF SELECTION

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INTRODUCTION

LUSH has recently pointed out that before applying the fundamental facts concerning the inheritance of productivity in farm livestock, measurements are needed of the magnitude of the forces involved. He is of opinion that "neglect to measure and integrate properly these variables is the major cause for the mistakes and unsound proposals that are sometimes made, even by those whose knowledge of biological fundamentals is not seriously deficient". He directs attention to two simple facts, the significance of which has not always been appreciated. "The first is that natural fertility and longevity set serious limits to the intensity of the selection which may be practised." "The second...is that such intensity is weakened (much more than is generally realised) by the inclusion of more and more items in the ideal; that is, by considering many different characteristics in making the selections." Lush goes on to say that here lies, he thinks, the only general basis of real antagonism between breeding for production and breeding for "fancy points", namely, that "each additional point considered must necessarily weaken the selection which might otherwise have been practised".

On reading Lush's paper it occurred to me that it might be worth while to examine with reference to intensity of selection certain flock records which some dozen years ago were very kindly placed at my disposal for another purpose by Mr G. Goland Robinson. These records cover the whole of a quarter of a century, from 1897 to 1922, from the founding of the flock to the dispersal sale, when Mr Robinson was in charge of the Wensleydale flock of Lord Henry Bentinck at Underley Farm near Kirkby Lonsdale. The examination of these records some years ago was the first part of an investigation of colour inheritance in the Wensleydale breed. This flock is a good one in which to investigate intensity of selection because every care was taken of the sheep, and it was more important to utilise good ewes to the full than to keep depre-

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ciation on female breeding sheep at a minimum. The ages up to which all ewes were retained whose ages of entering the flock were recorded are shown in Table I.

The Wensleydale breed is for two reasons a good one for the study of the intensity of selection. On the one hand, the breed is outstandingly fertile. On the other hand, the accepted colour standards being what they are, a big proportion of the lambs born, not merely the blacks, but an appreciable percentage of the whites, have on this account alone no chance of being chosen for breeding. Colour inheritance, therefore, as well as fertility, bears on intensity of selection.

I. COLOUR INHERITANCE

(1) *Wool colour types and their genetic relationship*

The Wensleydale wool colour types are white, black, and silver-grey. Black and silver-grey are sometimes conveniently grouped together as not-white or coloured. In coloured lambs the white fibres with tips intact always have "black", *i.e.* sepia, apical ends, though the pigment may be confined to the extreme tip and be visible only under the microscope. The percentages of white fibres to be stated were ascertained from counts at the basal end of the staple made at the age of about 3 months. Black lambs have always been found to possess a small sprinkling of white fibres, the darkest wool specimen having about a half of 1 per cent. Six per cent. is a frequent figure for black lambs. Lambs classed as silver-grey have from about 25 to 85 per cent. of white fibres on the main area of the fleece, with the extremities darker.

Earlier papers gave an analysis of the Underley records, with other facts and figures contributed by breeders, and the results of breeding experiments. White was concluded to be a simple dominant to coloured. It was suggested that silver-grey and black may differ by a single main factor, and that white, silver-grey, and black, in the order named, may form an epistatic series. When it is not necessary to distinguish between black and silver-grey, it is sometimes simplest to speak of white on the one hand and black on the other.

(2) *White versus coloured*

As published before, in matings between white rams and coloured ewes in which coloured lambs appeared in the progeny of every ram, less than half the lambs have been coloured. Additional matings make the totals:

White	75	Coloured	53
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The departure from a 1 : 1 ratio is not wide enough to be statistically significant.

The view that coloured is a simple recessive to white has been further tested by applying a very simple device to the Underley records, which had previously been analysed by the method of Sewall Wright. The proportion of white to not-white has been ascertained amongst all the offspring produced at later births by ewes proving their capacity to have black lambs. All lambs born at earlier births than the first black, or at the same birth as the first black, were excluded, and so were any lambs sired by the one ram (Lunesdale Quality) who could be regarded as a proved begetter of nothing but whites. The figures are:

White	646	Coloured	213
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The ratio is almost exactly 3 : 1.

(3) *Ear colour*

The significance of ear colour has been explained in the earlier papers, and more will be said about this aspect of colour inheritance in connection with intensity of selection. The breed standard calls for the skin of the face and ears to be deep blue in colour, and the more blue there is on other parts of the body, the more admired is the coloration. It is possible to state that, on the average, homozygous whites are lighter in colour inside the ears than heterozygous whites. It is only inside the ears that such a difference between the genotypes has been detected.

Sheep spoken of as "blue" are to be understood to be so coloured inside the ears. Amongst "pale" animals there are degrees of paleness. Most pales are more or less blue on the outside of the ears, but coppery within. Other pales are more lacking in pigment, a few being completely pink in complexion, including the outside and inside of the ears.

From the simple fact that 20.8 per cent. of all the Underley lambs, excluding those sired by Lunesdale Quality, were black (or silver-grey), it is calculated that approximately 83 per cent. of the breeding ewes were heterozygotes and the remaining 17 per cent. homozygotes. Over the 25 years at Underley there is no significant reduction in the percentage either of blacks or of pales (1924, p. 208). From an examination of the total performance of individual rams it is concluded that in years when the percentage of pales was unusually low, this is to be regarded as at least partly fortuitous, but possibly it is in part due to modifying factors carried by a small number of rams, who, while they sired an average proportion of blacks, had very few pale offspring.

Blue sheep mostly heterozygous whites. Of animals typically blue inside the ears it is clear that the large majority are heterozygous whites. This is the obvious interpretation of these three simple facts about the Underley flock: (1) There has been no reduction in the percentage of blacks born. (2) Blacks have never been allowed to breed. (3) Selection for the blue complexion has been as keen as practicable.

Here may be mentioned that of seventeen rams (only one of them had been used or bred at Underley) shown by their breeding performance to be homozygous whites, which I was able to inspect, only one was really blue inside the ears, another was fairly blue, and the rest were coppery. A few other rams, sires of nothing but whites, about which inquiry was made long after they were dead, were stated by breeders to have been typically blue, but the great majority of blue rams used in pedigree flocks have been heterozygous whites.

Blue sheep sometimes homozygous whites. That occasional blue rams are homozygous whites has been stated in the last paragraph. The standards for ear colour are perhaps more lenient for ewes than for rams. At Underley, as already stated, about one-sixth of the breeding ewes must have been homozygous whites. Six-sevenths of these breeding ewes, numbering 531, were bred at Underley, and only nine of these were classed as pale at birth. These nine probably became darker inside the ears with time. Five of them, by having black lambs, proved to be heterozygous whites.

In this same connection it may be noted that while approximately 29 per cent. of the lambs born must have been homozygous whites, only just over 13 per cent. were graded pale. It is calculated (from the percentage of 20.8 per cent. of blacks) that not more than approximately 76 per cent. of the lambs classed as blue were heterozygous whites. In view of the fact that a larger percentage than this of the breeding ewes in the flock, namely 83 per cent., are calculated to have been heterozygous whites, it may be suggested that selection for intensity of colour amongst the blues has been favourable to heterozygous whites.

As further evidence that sheep regarded as acceptably blue may be homozygous whites, there is the fact that homozygous white rams functioning in pedigree flocks have sired families of lambs much less than half of which were classed as pale.

Pale sheep sometimes heterozygous whites. In an experiment reported in an earlier paper a black ram mated with six pale ewes sired eighteen lambs, of which two, both from the same ewe, were black. This ewe was the palest of the six, her ears being pink outside and inside.

In breeding work brought to a close upon my leaving England for New Zealand, seven pale rams were tested by being mated with little flocks of black ewes. These rams had a good chance to be homozygous whites, for they were bred in the following way. Their mothers were old ewes, pale inside the ears, which in pedigree flocks had produced five or more lambs all whites by rams known to be heterozygous whites. The ewes were bought by the University of Leeds and mated to homozygous white rams. Of the seven pale rams so bred, five sired from nine to seventeen lambs, all whites, out of black ewes, but two, siring blacks, proved to be heterozygous whites.

In the breeding experiments on colour inheritance quite a number of lambs were produced, which, having one black parent, were heterozygous whites, and of these some few were pale. The proportion of lambs classed by myself as pale was in fact not significantly different from the proportion so classed in the Underley flock.

The complexity of the genetics of ear colour. Modifying factors, we must suppose, play a large part in the determination of blue ear colour. The same modifying factors are to be regarded as giving deeper blue in heterozygous than in homozygous whites.

Black sheep have black ears, and there is probably no visible difference whether the array of modifying factors be such as to tend, in white sheep, to more blue or to less blue, and this may afford the explanation of why some few of my white lambs that had one black parent were pale inside the ears.

Were the palest sheep picked out from the lambs of pedigree flocks, bred from parents chosen as far as feasible for deep blue complexion, it is likely that a high proportion of homozygous whites would be selected. From the fact that at Underley 20·8 per cent. of the lambs born were black, and from figures to be presented in the third section of this paper, the distribution of heterozygous and homozygous whites amongst the ewe lambs bred at Underley and kept for breeding, and those not retained (including a certain number that died), is calculated to be as follows:

	Retained for breeding	Not retained for breeding
Heterozygotes	441	250
Homozygotes	90	314

Of the 564 rejected ewe lambs, 162 were never given a flock number because they were deemed too pale.

It is my belief that it is possible to pick out heterozygotes by inspection with greater certainty than homozygotes. The problem of dis-

tinguishing definitely between the two white genotypes is unsolved. Indeed it has not been tackled.

II. FERTILITY

(1) *Lambs in multiple births not uniovular in origin*

Pairs of twins of opposite sex are equally as numerous as pairs of twins of the same sex. This has been ascertained from the twins with the two members of the pair of the same colour, that is, both black, both white-wooled with deep blue complexion, or both white-wooled with pale complexion. The numbers are:

Same sex	292 pairs	Opposite sex	291 pairs
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Amongst white twins in which the complexion of one member is called blue, and that of the other pale, the figures are:

Same sex	88 pairs	Opposite sex	78 pairs
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Amongst triplets with the three lambs all white or all black, seventy-four sets contained two lambs of one sex and one of the other, and in twenty-seven sets all three lambs were of the same sex, a proportion not far removed from 3 : 1, the ratio expected if each lamb was derived from a separate egg.

(2) *Age of ewe and fertility*

Several authors have published data on the fertility of ewes at different ages. The tables given by E. Roberts, Wriedt, and Jones and Rouse point to an increase in fertility up to 4 or 5 years, and to fertility being well maintained to the greatest ages at which it is customary to retain sheep in the flock. The figures now presented (Table I) show what is to be regarded as really greater fertility at 4 and 5 years than at 2 and 3 years, due partly to a significant decrease in barrenness and abortion (considered together), but at 4 years the average for all the ewes actually producing lambs shows a significant increase. It was found on analysing the ewe records that the ewes kept for more than 3 years were not significantly more fertile at 2 and 3 years than those not kept longer than 3 years. Beyond 5 years it is not possible to say more than that fertility was clearly well sustained.

The high figure for the small number of yearling ewes is attributed to special care before and during the mating season. Even in this fertile breed very few doubles are born at that age with less special management. In my experiments on colour inheritance, with a view to saving time, ewes were put to the ram in their first autumn when about 8 months old.

From forty-eight such animals the result was seventeen singles and thirty-one blanks.

The table shows that 69 per cent. of all lambings were multiple births. A total of 1524 lambings gave 468 single lambs, 869 pairs of twins, 183 sets of triplets, and four fours. Save for animals put to the ram in their first year, flushing the ewes for mating was generally avoided. In the Wensleydale breed, as a breeder expressed it, there is a danger of flushing leading to more triple than single births.

TABLE I
Age of ewe and fertility

Age of ewe years	No. of ewes put to the ram	No. of ewes barren or aborting	% ewes barren or aborting	Total lambs	% multiple births (calculated on total births)	Average no. of lambs per ewe having lambs. Average \pm standard error	Average no. of lambs per ewe put to the ram. Average \pm standard error
1	31	Not recorded	—	58	74	1.81	—
2	539	53	10	849	66	1.75 \pm 0.03	1.58 \pm 0.03
3	419	28	7	699	67	1.79 \pm 0.03	1.67 \pm 0.03
4	320	11	3	606	78	1.96 \pm 0.04	1.89 \pm 0.04
5	189	5	3	346	70	1.88 \pm 0.05	1.83 \pm 0.05
6	77	1	—	135	67	1.78 \pm 0.07	1.75 \pm 0.07
7	32	1	—	55	65	1.77 \pm 0.12	1.72 \pm 0.13
8	11	0	—	19	—	—	—
9	3	0	—	6	—	—	—
10	1	0	—	2	—	—	—
11	1	0	—	2	—	—	—
On total figures:							
	1623	99	6	2775	69	1.82	1.71
On total figures, excluding ewes of 1 year:							
	1592	99	6	2719	69	1.82	1.71

(3) *Inheritance of fertility within the Underley Wensleydale flock*

In other breeds of sheep there is evidence for differences of innate fertility within the flocks studied. Wriedt, for example, working on Cheviot sheep, compared the fertility of mothers and the fertility of daughters. He placed the first generation ewes ("mothers") into groups according to their average number of lambs at a birth, and then, for each such group, ascertained the average number of lambs at a birth of the ewes of the second generation ("daughters"). He gave the following figures:

Mothers	Daughters
No. of lambs at a birth	Average no. of lambs at a birth
1	1.30
1.1-1.3	1.36
1.4-1.6	1.43
1.7-1.9	1.59
2 or more	1.72

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The result of an analysis of the Underley records by Wriedt's method is given in Table II (a) and (b).

TABLE II

Inheritance of fertility within the Underley Wensleydale flock

The "mothers" included all had at least three crops of lambs.
All the same sheep figure in (a) and (b).

(a) Averages calculated on the number of crops of lambs actually produced.

Grouping on no. of lambs at a birth	Mothers				Daughters			
	No. of ewes	No. of births	No. of lambs	Average no. of lambs at a birth	No. of ewes	No. of births	No. of lambs	Average no. of lambs at a birth
1.0-1.2	7	28	31	1.11	8	18	35	1.94
Above 1.2-1.4	14	50	66	1.32	21	64	116	1.81
" 1.4-1.6	18	80	123	1.54	28	83	153	1.81
" 1.6-1.8	52	201	347	1.73	78	207	373	1.80
" 1.8-2.0	43	189	376	1.99	91	239	436	1.82
" 2.0-2.8	49	202	473	2.34	82	236	430	1.82
All groups	183	750	1416	1.89	308	847	1543	1.82

(b) Averages calculated on the number of years in which a ewe was put to the ram.

Mothers		Daughters	
Grouping on no. of lambs per breeding season		Average no. of lambs per breeding season	
1.2 or less		1.86	
Above 1.2-1.4		1.82	
" 1.4-1.6		1.73	
" 1.6-1.8		1.73	
" 1.8-2.0		1.76	
" 2.0-2.8		1.77	

The table shows that whether the mothers have a low or high average, the daughters on the average are equally fertile. The simplest explanation is that differences in the fertility of ewes are fortuitous or due to non-genetic factors, and that the whole flock stands at a uniformly high level for inborn fertility. Figures in Table III show high fertility in other Wensleydale flocks. The breed is numerically a small one and most of the rams used in pedigree flocks are bred by only a few breeders. The whole breed is probably pervaded with innate high fertility.

(4) *Fertility in Wensleydale crosses*

The function of the Wensleydale breed in British sheep husbandry is to provide rams for crossing with mountain breeds, like Scots Blackface, Kendal Rough, and Swaledale, and for crossing with Wensleydale-Mountain cross-bred ewes. Table III is compiled from figures kindly supplied by a number of breeders in the north of England.

TABLE III

Inheritance of fertility in Wensleydale crosses

Mating	No. of ewes put to the ram	% ewes geld or aborting	Average no. of lambs per ewe put to the ram
Pure Wensleydales:			
Underley flock, 1898-1922 (date of Table I)	1623	6	1.71
Various flocks, 1927	644	5	1.76
Pure Mountain breeds (Scots Blackface, Kendal Rough, and Swaledale) 1927	1815	7	1.00
Wensleydale rams × Mountain ewes, 1927	2960	5	1.29
Wensleydale rams × Wensleydale-Mountain cross-bred ewes, 1927	956	3	1.76

The difference between the figures for Mountain ewes put to rams of their own breed and Mountain ewes put to Wensleydale rams is not to be credited to the Wensleydale rams, but is to be explained simply by better feed and physical conditions. In the same way some allowance must be made for non-genetic factors when considering the high figures from Wensleydale-Mountain cross-bred ewes, but comparison with figures for a variety of British breeds published by Heape and by Nichols shows that this figure is so extremely high that it is reasonable to postulate one or more dominant genetic factors for high fertility transmitted by the Wensleydale rams in the first cross.

III. INTENSITY OF SELECTION

(1) *Selection of heterozygous whites*

First will be considered the selection of heterozygous whites as breeding ewes. It has already been made clear that at Underley a substantial preference was shown to heterozygotes, and this is brought out further in Table IV. In other pedigree flocks from which figures were

TABLE IV

Selection of heterozygous whites

Flocks	% black lambs born	Calculated % of heterozygous whites in the breeding ewes	Calculated % of heterozygous whites in white lambs born	Calculated % of homozygous whites in white lambs born
Underley	20.8	83.2	63.1	36.9
Other flocks	15.7	62.8	59.3	40.7

collected the percentage of black lambs born was less than at Underley, being 15.7 per cent. on 3735 lambs. On the assumption that all the rams were heterozygous whites simple calculations give the figures put down

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in the table. It will be seen that in the other flocks differential selection in favour of heterozygotes was much less than at Underley. The records show that at Underley the proportion of heterozygotes was high in the original sheep purchased, and that this proportion has been consistently preserved by selection down the whole quarter of a century.

(2) *Proportion of female lambs retained for breeding*

The number of breeding ewes acquired from other flocks was only eighty-eight, and all but thirteen were bought before 1905 when the numbers were being increased from four (1898) and twenty-four (1899) to seventy-four (1905), the approximate size maintained ever afterwards.

The following are the numbers of ewe lambs of the different colour types born from the foundation of the flock up to 1920, the lambs of that year being the last which had the chance to become members of the breeding flock. The sex, and sometimes the colour, of 107 lambs dead at or soon after birth was not recorded, but very frequently the sex and colour of dead lambs were stated in the records:

White, of blue complexion	895
White, of pale complexion	200
Black	263
Total			1358

Of the lambs classed at birth as pale, thirty-eight were later given a flock number. This meant that they were deemed passable on the score of ear colour, which sometimes becomes darker as the lamb gets older. Of these thirty-eight lambs, as stated earlier, nine became breeding ewes, and five of them had black lambs. Of the white ewe lambs of the Underley flock we have therefore:

Approved on complexion	933
Rejected on complexion	162

Adding together the pales and the blacks we have:

Approved on colour	933, being 68·7 per cent.
Rejected on colour	425, being 31·3 per cent.

Of the ewe lambs bred at Underley, 531 were selected for breeding ewes. This is 39·1 per cent. of the female lambs born, excluding the dead lambs of unrecorded sex, or 37·6 per cent. if half of those dead lambs are added to the total of ewe lambs. Of the female lambs recorded as acceptable for colour, 56·9 per cent. found their way into the breeding flock.

(3) *The average family of a ewe*

The number of ewes, whether bought from other flocks, or bred at Underley, known to have their first lambs in the Underley flock, and in due course, in whatever way, or for whatever reason, passing out of the flock, was 493. They had 2521 lambs, including all dead ones, an average of 5.11 lambs a ewe.

As stated earlier, the average number of lambs per mating was 1.71. This means that on the average a ewe figures in the breeding flock for three seasons.

Slightly more ewe lambs than ram lambs were born, the figures for the 25 years being 1485 ♀♀, 1423 ♂♂, with the sex not recorded for 107 lambs. Allowing for this sex ratio, each ewe on the average produced 2.61 female lambs, of which one could be expected to be kept to replace the mother. That would mean the retention of 38.3 per cent. of all the ewe lambs born, a figure essentially the same as the percentage of ewe lambs born at Underley which actually did enter the breeding flock.

(4) *The Wensleydale colour standard and the intensity of selection*

There is no need to enlarge upon the limitation placed on the intensity of selection by the adoption of a colour standard in deference to which the selection of heterozygous whites is practised.

It is true, as shown in this paper, that the Wensleydale breed is exceptionally fertile, but to bar, on the score of colour, 30 per cent. of the lambs born, means that the scope of selection in female breeding sheep is no better than in a breed of very moderate fertility.

SUMMARY

1. This paper has been stimulated by recent illuminating remarks by Lush about the inescapable limits to intensity of selection amongst females in farm livestock.

2. Most white Wensleydale sheep, deep blue inside the ears, are heterozygous whites. Many animals pale inside the ears, but by no means all, are homozygous whites. The significance of ear colour in white Wensleydale sheep is discussed more fully than in earlier papers.

3. Monozygotic twinning, if it ever occurs, is very rare.

4. Fertility is high in the Wensleydale breed. The average number of lambs per ewe put to the ram at Underley was 1.71. Of all the lambings 69 per cent. were multiple births.

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5. Fertility is higher at 4 and 5 years than at 2 and 3 years, this being partly due to decrease in barrenness and abortion.

6. Within the Underley flock differences of inborn fertility were searched for in vain. It may well be that the whole breed stands at a high level of innate fertility.

7. In Wensleydale crosses high fertility is transmitted by the Wensleydale rams to their cross-bred daughters.

8. At Underley nearly 40 per cent. of all ewe lambs born were retained in the flock as breeding ewes.

9. Ewes remained in the flock, on the average, for three seasons, and produced 5.11 lambs.

10. The Wensleydale colour standard severely limits the intensity of selection for non-colour characters which can be practised amongst female breeding sheep.

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THE PARENTS AND PROGENY OF *AESCULUS CARNEA*

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(With Plates I and II and Twenty-four Text-figures)

INTRODUCTION

AESCULUS CARNEA arose more than a century ago presumably as a chance hybrid between *Ae. Hippocastanum*, the European horse chestnut, and *Ae. Pavia*, the North American buckeye. *Ae. Hippocastanum* grows to a height of 80–100 ft., has resinous buds, white flowers and prickly fruits. Its five or seven large leaflets are sessile upon the petiole. *Ae. Pavia*, once placed in a separate genus *Pavia*, is a shrub never taller than 18 ft. with non-resinous buds, red flowers and smooth fruits. It has five leaflets, all of which are stalked. The characters of the hybrid are intermediate between those of the two parents (Plate I a). *Ae. carnea* is a tree of about 30–40 ft., its buds slightly resinous, its flowers pink and its fruits somewhat spiny. The leaflets are intermediate in size between those of *Ae. Hippocastanum* and *Ae. Pavia* and are attached to the petiole by very short stalks. Although it is somewhat less fertile than *Ae. Hippocastanum*, its seeds germinate readily and it breeds true (Bean, 1914).

The explanation of this unexpected behaviour lies in the fact, discovered by Hoar (1927), that *Ae. carnea* is tetraploid, having 80 chromosomes, while all the other species of the genus have 40. This account was confirmed by Skovsted (1929). He points out that the new species was derived presumably by the doubling of a sterile hybrid, as in the classical case of *Primula kewensis*. But he goes further. He states that the complement of *Aesculus carnea* is made up of 40 large chromosomes derived from *Ae. Pavia* and 40 small ones from *Ae. Hippocastanum* which can be distinguished from one another at the first metaphase of meiosis. A similar case has been described by the same author in cotton (Skovsted, 1935).

There are many examples of chromosomes of different sizes derived from different parents pairing at meiosis. In these cases the partners differ structurally, that is, one has gained or lost segments by structural

change in the course of its history. But where two species, like the parents of *Ae. carnea*, differ, as Skovsted states, uniformly in size, no such explanation is possible. The one cannot have uniformly lost part of each chromosome, nor can the other have uniformly gained. Such a difference must be genotypically controlled unless it is determined by some special "accessory substance" in the chromosomes themselves (Darlington, 1932 a). Many cases of hybrids and mutants showing this genotypic control of size are known. For example, Navashin (1931) describes a hybrid between *Crepis capillaris* and *C. neglecta* in which the chromosomes derived from *C. capillaris* are longer and those from *C. neglecta* shorter than they are in the parental species. Similarly a triploid *Tradescantia*, presumably a hybrid between the tetraploid *T. virginiana* with large chromosomes and a diploid species with smaller ones, had chromosomes as large as those of the tetraploid, with the exception of one bud, in which the chromosomes and the nuclei were reduced by mutation to one-fifth of their normal size (Darlington, 1929 b). It is an essential property of these hybrids that genotypically controlled differences between the chromosomes of the parents disappear in the hybrid when they are brought under a new and uniform genotypic control.

Aesculus carnea is therefore, according to Skovsted's description, an exception to a general rule, and as such calls for specially careful examination. For this reason I undertook to reinvestigate it, together with its parents and its sterile derivative *Ae. plantierensis*.

MATERIAL AND METHODS

The anthers are small at the time of meiosis, and the pollen mother cells stick together somewhat; yet I found that smearing was the most satisfactory method of obtaining preparations. In *Ae. Pavia* the pollen mother cells are more difficult to smear than in the other species, and the division very rapid, so that very few metaphases could be obtained. In this species therefore, in addition to making smears, I dissected out the anthers and fixed them for embedding.

Root tips were obtained by taking the lateral roots from freshly germinated chestnuts. The main root is useless on account of the accumulated starch and its great size, which prevents rapid penetration of the fixative. Anthers were cut at 10μ and root tips at 6μ .

All preparations were fixed in 2 BE (La Cour, 1931) and stained in Newton's gentian violet. In staining the pollen mother cells of *Ae. Pavia* and the root tips of all species, it was necessary to leave the slides in the

gentian violet for half an hour and mordant in iodine for about 10 min. before passing rapidly through the alcohols into clove oil.

The material examined came from the following sources:

Species	Root tips	Pollen mother cells
<i>Ae. flava</i> $2n=40$	Garden of Sir George Cooper, Winchester	Royal Botanic Gardens, Kew
<i>Ae. Pavia</i> $2n=40$ (1)*	Botanic Garden, Copenhagen	—
(2)	Royal Gardens, Windsor	—
(3)	—	Royal Botanic Gardens, Kew
<i>Ae. Hippocastanum</i> $2n=40$	John Innes Hort. Inst., Merton	John Innes Hort. Inst., Merton
<i>Ae. carnea</i> $2n=80$	John Innes Hort. Inst., Merton	John Innes Hort. Inst., Merton
<i>Ae. plantierensis</i> $2n=60$	—	Royal Botanic Gardens, Kew

* There appears to be some doubt as to the identity of this plant. It is probably *Ae. flava*.

Ae. plantierensis is a sterile species presumed to be a back-cross between *Ae. carnea* and *Ae. Hippocastanum* which was raised in the nurseries of Messrs Simon-Louis Frères at Plantières near Metz (Bean, 1914).

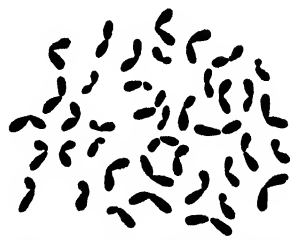
MITOSIS AND MEIOSIS

The genus *Aesculus* is divided into two groups on morphological grounds, the Eu-Aesculus section and the Pavia section. The former, to which *Ae. Hippocastanum* belongs, has, according to Skovsted, chromosomes about one-eighth the size of those of the Pavia section, of which *Ae. Pavia* and *Ae. flava* are members. I find no such difference either in the somatic divisions (Text-figs. 1-5) or at meiosis (Text-figs. 6-11). There are, however, slight differences in size within each complement, and these are comparable. The largest chromosomes have median centric constrictions and are about $1.3-1.5\mu$ in length. The smallest have sub-terminal constrictions and are about $0.5-0.6\mu$. Measurements of this order are necessarily only of comparative value, since the wave-length of green light used for observation is itself 0.5μ . Precisely similar differences occur within the complement of *Ae. carnea* (Text-fig. 5). Furthermore in spite of their small size it was possible to pick out two chromosomes with trabants, two with very long centric constrictions and two with secondary constrictions. These constrictions are probably associated with the formation of the nucleoli (cf. Text-figs. 6, 7). Probably such chromosomes also occur in one or other of the parental species, but the preparations were not sufficiently critical to show such fine details

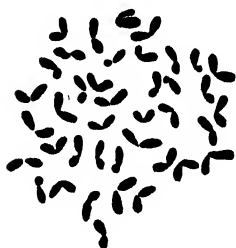
Metaphases flat enough to photograph could be found only in *Ae. Hippocastanum* and *Ae. Pavia* (Plate I b).



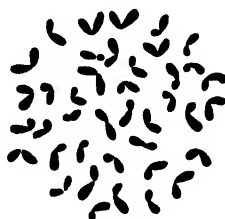
Text-fig. 1. *Ae. flava*
 $2n=40$



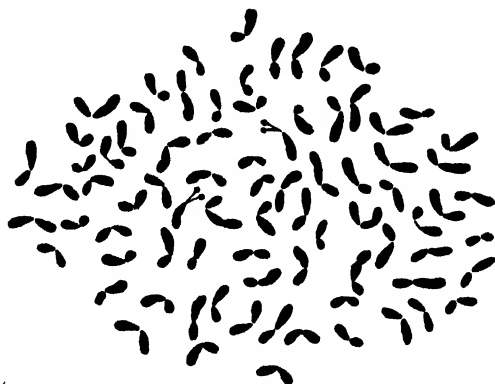
Text-fig. 2. *Ae. Hippocastanum*
 $2n=40$



Text-fig. 3. *Ae. Pavia* $2n=40$
(from Windsor)



Text-fig. 4. *Ae. Pavia* $2n=40$
(from Copenhagen)



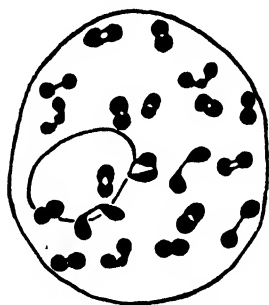
Text-fig. 5. *Ae. carnea* $2n=80$

Text-figs. 1-5. Somatic divisions from the root tip. $\times 4800$.

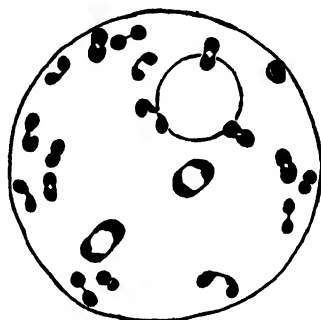
It may be noted parenthetically that the type of nucleus corresponds to that found in the Cruciferae (Manton, 1935), that is to say, it is of the "solid" type with a single central fusion-nucleolus. In organisms with large chromosomes the two or more nucleoli which are formed at telo-

phase remain distinct until the following prophase. It is possible to distinguish a polyploid from the related diploid by the number of nucleoli in the resting nuclei (*Hyacinthus* de Mol, 1928; *Crepis* Geitler, 1932). In *Aesculus carnea*, however, this is not so. At telophase several nucleoli can be seen in the process of forming, but the resting nuclei look exactly like those of its parents.

At meiosis no differences in size can be detected within the complements owing to the greater degree of contraction and the presence of chiasmata. The only difference between *Ae. Hippocastanum* and *Ae. Pavia* at diakinesis (Text-figs. 6, 7) is the difference in nuclear size. This is, however, possibly due to differences of fixation, since one was taken from a smear and the other from a section.



Text-fig. 6. *Ae. Hippocastanum*, (Smear)
33 chiasmata



Text-fig. 7. *Ae. Pavia* (Section),
27 chiasmata

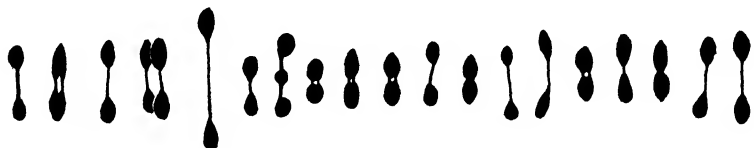
Text-figs. 6, 7. Diakinesis. $\times 4800$. Two bivalents are associated with the nucleolus in each cell.

Terminalisation is usually complete, though there is some variation from cell to cell (Text-figs. 8–11). The differences are local and affect parts of the anther and not simply individual cells. In a smear of *Ae. Pavia* I found no interstitial chiasmata (Text-fig. 10), while in one part of an anther in a section, nearly every cell had one or more (Text-fig. 11).

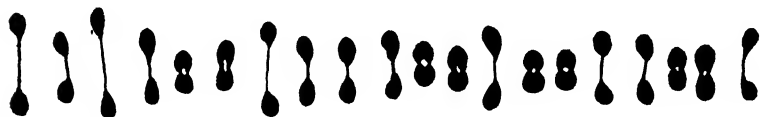
With slightly larger chromosomes, such as those of *Lycopersicum esculentum* or *Primula sinensis*, a distinction can be made between a loop containing two chiasmata and two free ends, but here it becomes impossible. Hence the cells with a number of apparently interstitial chiasmata may really be cells with a higher chiasma frequency, such that there are two chiasmata in one chromosome arm instead of one or none. The presence of a distal chiasma would prevent the proximal one from terminalising and would account for the difference observed. The

numbers of chiasmata have been estimated on this assumption. Since the preparations were made on separate occasions, it is probable that some external factor, such as temperature, was a cause of the difference.

Although polar views of metaphase are less valuable than side views for estimating the number of chiasmata, they nevertheless reveal relationships quite impossible to detect from side views. We see (Text-figs. 12-15 and Plate II) in each of the so-called diploids a marked degree of



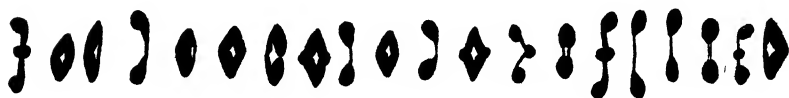
Text-fig. 8. *Ae. flava* showing a ring quadrivalent, 31 chiasmata



Text-fig. 9. *Ae. Hippocastanum*, 29 chiasmata



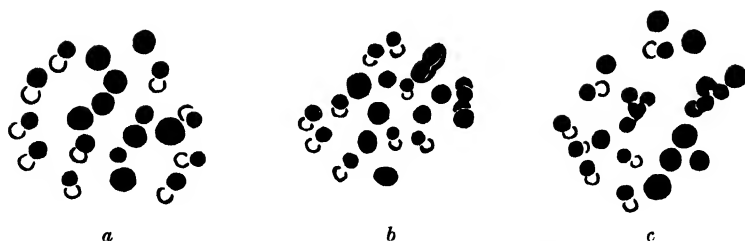
Text-fig. 10. *Ae. Pavia* (Smear), 29 chiasmata



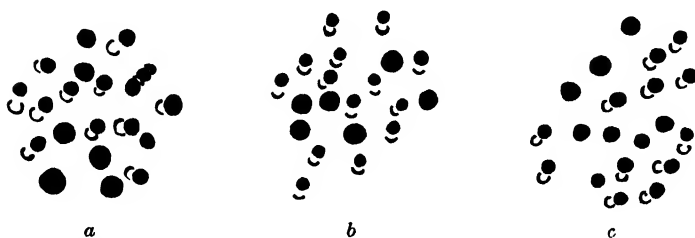
Text-fig. 11. *Ae. Pavia* (section), 31 chiasmata. Several bivalents have two chiasmata in the same arm

Text-figs. 8-11. Side views of first metaphase of meiosis. $\times 4800$.

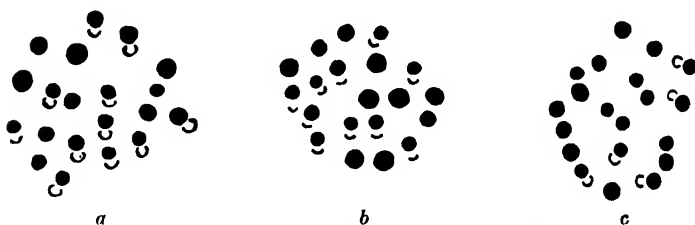
secondary pairing (Darlington, 1928). Indeed from Skovsted's illustrations, Darlington (1932 *b*, p. 218) calls *Ae. Hippocastanum* and *Ae. Pavia* "tetraploids", and *Ae. carnea* an "octoploid". The assumption of tetraploidy in the parental species is borne out by the formation of an occasional quadrivalent (Text-figs. 12, 13), not only in *Ae. Hippocastanum* but also in related species. No quadrivalents were observed in *Ae. Pavia* (Text-fig. 14), where secondary pairing is less marked (Plate II). Rather more occur in the octoploid hybrid than in *Ae. Hippocastanum*, proving that the chromosomes from *Ae. Pavia* also take part in their formation



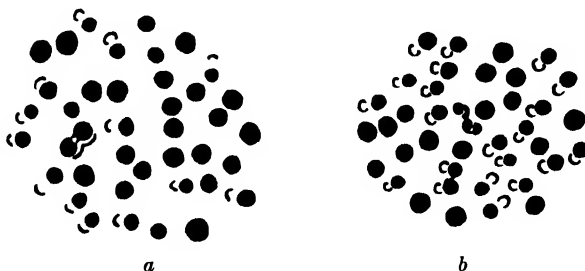
Text-fig. 12. *Ae. flava*, *a*, 10 rods, 10 rings; *b*, 2 quadrivalents, 9 rods, 7 rings;
c, 2 quadrivalents, 7 rods, 9 rings



Text-fig. 13. *Ae. Hippocastanum*, *a*, 1 quadrivalent, 11 rods, 7 rings;
b, 14 rods, 6 rings; *c*, 12 rods, 8 rings



Text-fig. 14. *Ae. Pavia*, *a*, 11 rods, 9 rings; *b*, 10 rods, 10 rings; *c*, 5 rods, 15 rings



Text-fig. 15. *Ae. carnea*, *a*, 1 quadrivalent, 13 rods, 25 rings;
b, 2 quadrivalents, 19 rods, 17 rings

Text-figs. 12-15. Polar views of metaphase of the first division, showing secondary pairing. $\times 4800$. The rods have one, the rings two chiasmata.

(Text-fig. 15). It is to be noted that all four chromosomes are the same size.

Polar views also cast some light on Skovsted's size differences. There are indeed size differences within the complement of *Ae. carnea* (Text-fig. 15) at this stage, as he shows, but they are due, not, as he believed, to differences in the origin of the chromosomes, since there is no difference between the parents, but to differences in the number and position of chiasmata between the bivalents (see Diagram 1). The apparently small bivalents have only one chiasma and consequently appear almost spherical. The large bivalents have two chiasmata, which bend them

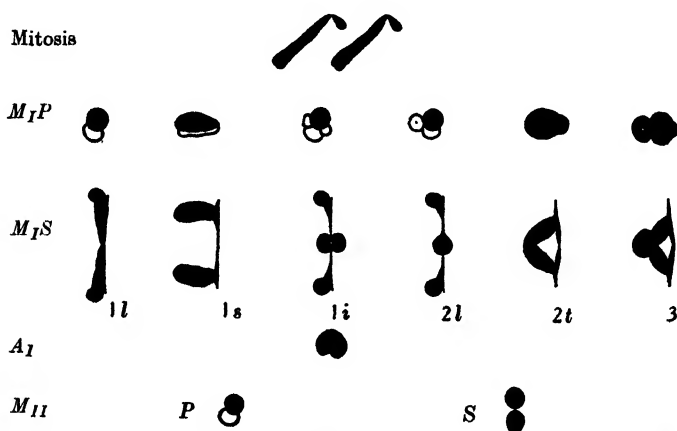
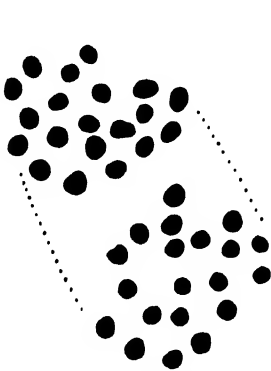
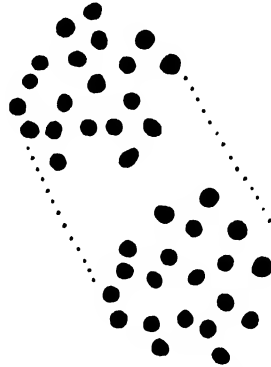
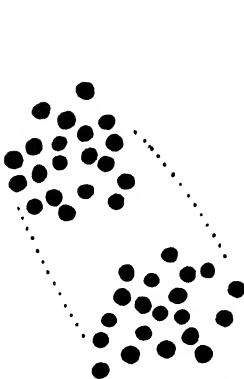
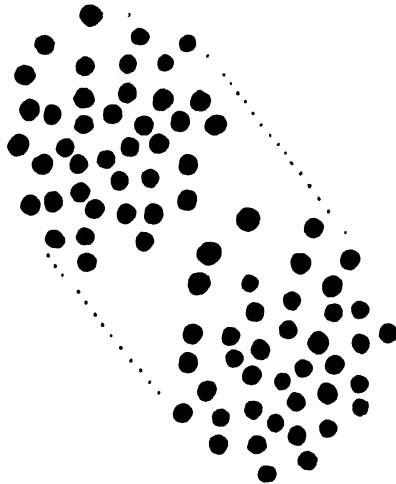


Diagram 1. Showing the effect of chiasmata on apparent chromosome size. $M_I P$, polar view of the first meiotic metaphase. $M_I S$, side view of the first meiotic metaphase. $1 l$, one chiasma in long arm. $1 s$, one chiasma in short arm. $1 i$, interstitial chiasma in long arm. $2 l$, two chiasmata in the long arm. $2 t$, two terminal chiasmata, one in each arm. 3 , three chiasmata, two in the long arm, one in the short.

into a loop. Close examination of the plate itself shows that the "small" bivalents are on a higher and lower level, while the "large" ones occupy the middle region (cf. Text-figs. 8-11). Precisely similar differences occur in all the species.

This conclusion is borne out by a comparison of *Ae. flava* with the other forms. This species has chromosomes of the same size as its relatives, as seen at mitosis (Text-fig. 1). In polar views of metaphase of meiosis on the other hand (Text-fig. 12), it has more "large" chromosomes than the others; in side views (Text-fig. 8) it is seen to have more bivalents with two chiasmata, and consequently it forms more quadrivalents. In a word, the apparent differences in size are due to differences in chiasma frequency.

Anaphase of the first division is a more useful stage for detecting size differences, since the strain set up by the chiasmata has been released and the chromosomes are all in a similar state of relaxation as it were. The differences of size observed at mitosis are almost completely obscured

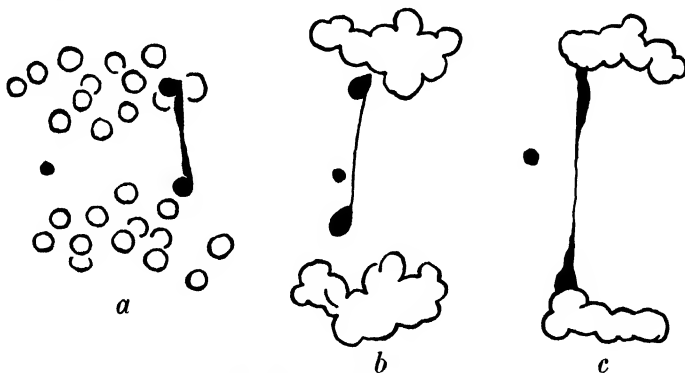
Text-fig. 16. *Ae. flava*Text-fig. 17. *Ae. Hippocastanum*Text-fig. 18. *Ae. Pavia*Text-fig. 19. *Ae. carnea*

Text-figs. 16-19. Polar views of anaphase of the first division. $\times 4800$.

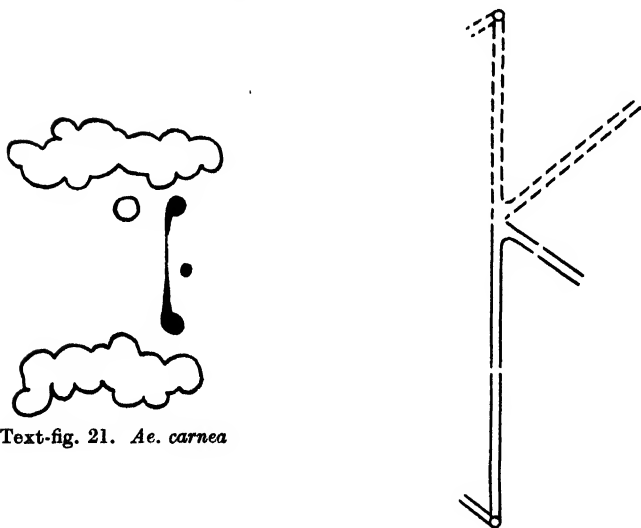
(Text-figs. 16-19). Secondary pairing is still maintained. Hoar's (1927) illustration (his Fig. 1) shows this very clearly in *Ae. carnea*.

Side views of anaphase show that the individual of *Ae. Hippocastanum* studied is structurally hybrid for part of at least one of its chromosome pairs. Somewhat infrequently, that is to say in 2 or 3 per cent. of the

anaphases observed, a bridge occurs together with a fragment, which remains lagging on the plate (Text-fig. 20). This is the result of crossing-



Text-fig. 20. *Ae. Hippocastanum*



Text-fig. 21. *Ae. carnea*

Text-fig. 22

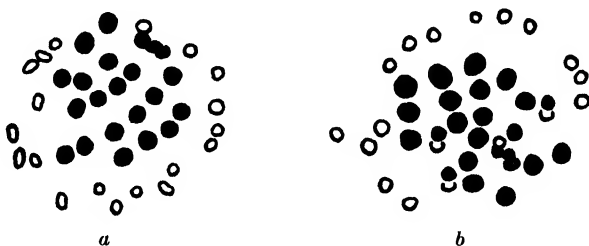
Text-figs. 20, 21. Side views of anaphase I. $\times 4800$. Showing bridges and acentric fragments.

Text-fig. 22. Diagram showing the structure of a bivalent with a single chiasma between relatively inverted segments of homologous chromosomes (from Darlington, 1935).

over between two relatively inverted segments in such a way that of the four chromatids, one is dicentric, one acentric and two monocentric or normal (see Diagram 1). In early anaphase (Text-fig. 20 *a* and *b*) it is possible to distinguish the chromosome in which this inversion is present.

At telophase it becomes obscured among the other chromosomes, leaving only a fine thread stretching across the plate (Text-fig. 20 *c*), which is usually broken later by the formation of the cell wall. A similar bridge was found in *Ae. carnea* (Text-fig. 21), but its occurrence was rarer. It presumably results from exceptional pairing between chromosomes of *Pavia* and *Hippocastanum*.

The pairing relationships of the parental species are revealed more completely by the back-cross of *Ae. carnea* to *Ae. Hippocastanum*-*Ae. plantierensis*. This hybrid is triploid relative to its diploid parents, or more correctly hexaploid, with 60 chromosomes, 40 having been received immediately from *Ae. carnea* and 20 from *Ae. Hippocastanum*. Originally therefore 20 come from *Ae. Pavia* and 40 from *Ae. Hippo-*



Text-fig. 23. Polar views of metaphase I of *Ae. plantierensis*. $\times 4800$. *a*, 1 quadrivalent, 19 bivalents, 18 univalents; *b*, 1 quadrivalent, 21 bivalents, 14 univalents

castanum. Yet the plant does not invariably form 20 bivalents and 20 univalents. In fact I have never observed this combination. Quadri-valents occur in most cells (Text-fig. 23) and again show that the supposed diploid parents are in fact tetraploids. With them occur varying numbers of bivalents and univalents. This behaviour is an example of the well-known property of differential affinity. Chromosomes which pair regularly in the presence of an identical partner, pair variably with other and dissimilar chromosomes to which they would never be attracted in the presence of more likely mates.

A few of the univalents lie off the plate towards the poles of the spindle, but the great majority of them arrange themselves at metaphase on the edge of the plate surrounding the bivalents. This behaviour is exactly similar to that found by Kihara (1929) in the *Triticum-Aegilops* hybrids.

CHROMOSOME MEASUREMENT AT MITOSIS AND MEIOSIS

The question of chromosome size has led investigators astray from the early days of cytology. Farmer and Digby (1914) attempted to prove that the chromosomes of the tetraploid *Primula kewensis* were at meiosis half the size of those of the diploid. Fifteen years elapsed before Newton and Pellew (1929) showed this finding to be incorrect. Again Tischler (1927) alleged that the chromosomes derived from the two parents of *Ribes Gordonianum* could be distinguished by their size at first meiotic metaphase and that there was complete autosynopsis. Darlington (1929 *a*) showed that the difference in size in the somatic divisions was not greater than that occurring within the complements of the parents, and that at meiosis normal pairing was taking place, not autosynopsis. More recently even mitosis has proved a pitfall; Ellison (1935) illustrates two varieties of potato, Golden Wonder and Langworthy, of which he says after elaborate measurement that the former has more long chromosomes than the latter. It has since been proved by Crane (1936) that Golden Wonder is a periclinal chimaera of which all but the surface layer is Langworthy. The roots of the two should therefore be identical and have been shown to be so (Upcott, *cit.* Crane, 1936).

The comparison of meiotic chromosomes in different organisms presents difficulties of an entirely different kind. The greater degree of contraction obscures slight differences of size which can be detected at mitosis. Furthermore, as we have seen, the same pair of chromosomes appears large or small according to the number and position of their chiasmata. In the genus *Aesculus* the differences within the complements of each species and of the hybrid are slight and cannot be detected at the first meiotic anaphase or at the second division. The apparent differences at the first metaphase of meiosis are due to differences in the arrangement of chiasmata and are similar in the hybrid and both the parents. It is possible that Skovsted has found a cell of *Ae. carnea* which had 20 bivalents with two chiasmata, apparently large, and 20 bivalents with one chiasma, apparently small, which he thinks have been derived from the two parents respectively.

His statement that the chromosomes of *Ae. Pavia* are larger than those of *Ae. Hippocastanum* is, however, more difficult to explain. It is possible that with his method of fixation (Carnoy) one group of species was given a different size from the other. This difference, of course, does not apply within the hybrid. That it has no validity is shown by the

uniform results I have obtained both at meiosis and mitosis in all the forms, parent, hybrid and derivative, which I have examined.

It now becomes clear that *Ae. carnea* is not an exception to the general rule of genotypic control, as it has been considered, since there is no size difference between the parents to be determined either structurally or genotypically.

SUMMARY

1. The somatic chromosomes of *Aesculus Hippocastanum* ($2n=40$) and of *Ae. Pavia* ($2n=40$) are exactly similar in size and shape, although each complement contains within it differences which are also distinguishable in the complement of the hybrid *Ae. carnea* ($2n=80$).

2. In both species, and in the hybrid, polar views of metaphase I show about half the bivalents larger than the rest, and this is due to their having chiasmata in both arms. This difference necessarily disappears at anaphase.

3. Secondary pairing and the formation of an occasional quadrivalent show the parent species to be tetraploid. The hybrid must therefore be regarded as octoploid.

4. *Ae. plantierensis* ($2n=60$) is a hexaploid back-cross of *Ae. carnea* \times *Ae. Hippocastanum*, and forms varying numbers of multivalents, bivalents and univalents.

5. The individuals of *Ae. Hippocastanum* and *Ae. carnea* examined are heterozygous for inversions.

ACKNOWLEDGMENT

I wish to thank Dr C. D. Darlington and Mr M. B. Crane for help and criticism during the course of this investigation.

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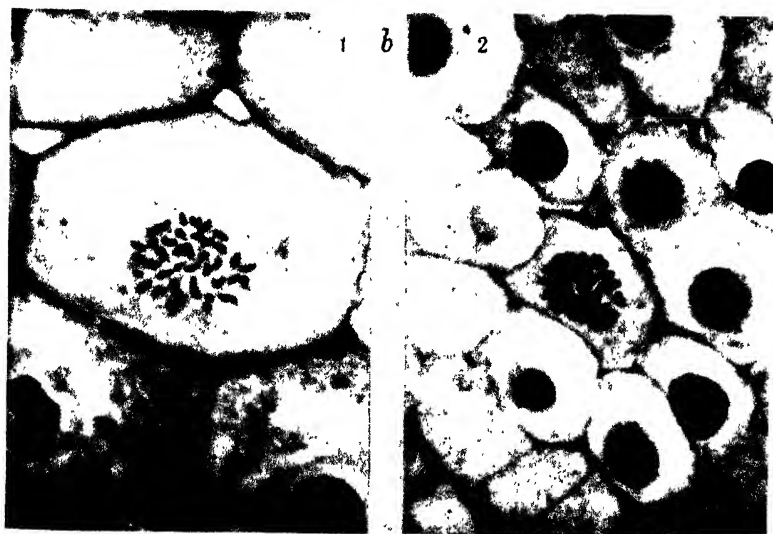
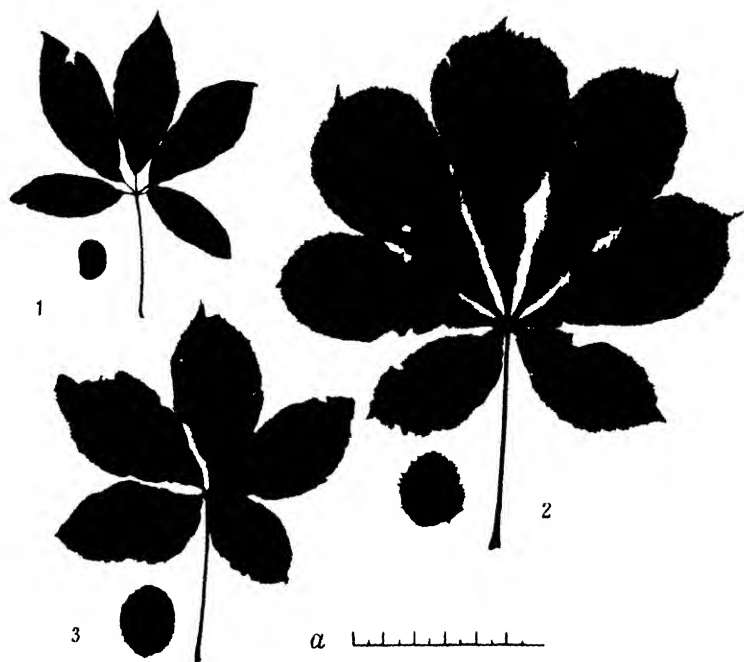
EXPLANATION OF PLATES I AND II

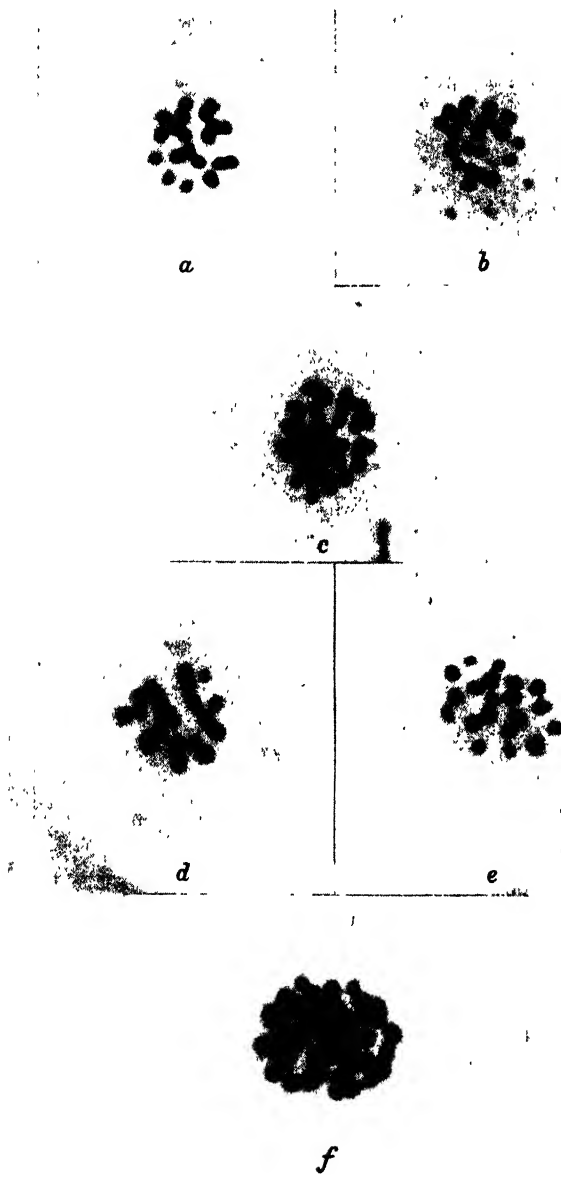
PLATE I

- (a) Leaves and fruit of the parents and hybrid showing the intermediate character of the latter. (1) *Ae. Pavia*; (2) *Ae. Hippocastanum*; (3) *Ae. carnea*.
- (b) Somatic divisions from the root tip of (1) *Ae. Hippocastanum*, (2) *Ae. Pavia*. $\times 2400$. The "solid" nuclei and prochromosomes can also be seen.

PLATE II

- Polar views of first metaphase showing secondary pairing. (a) *Ae. Hippocastanum*; (b) *Ae. Pavia*; (c) *Ae. carnea*; $\times 2400$. (d) *Ae. Hippocastanum*; (e) *Ae. Pavia*; (f) *Ae. carnea*; $\times 3200$.





APPENDIX

Since sending this article to the press, I have been able to compare the *Ae. Pavia* plants from the Royal Gardens, Windsor, and from the University Botanic Garden, Copenhagen, with the descriptions and illustrations in:

Loddiges Botanical Cabinet, 13, 1257.

Engler and Prantl, *Pflanzenfamilien*, III, 5, 276.

Britton and Brown, *Illustrated Flora of the Northern United States*, etc., 2, 500.

Rehder, *Manual of cultivated trees and shrubs*, p. 582.

The Windsor plant which I used is true *Ae. Pavia*. The Copenhagen plant, used by Skovsted, is *Ae. hybrida*, a hybrid between *Ae. octandra* and *Ae. Pavia*.

I am indebted to Mr Cook of the Royal Gardens at Windsor and to Dr Axel Lange, Curator of the Copenhagen Botanic Garden, for sending me seeds and flowers.

THE GENETICS OF *TROPAEOLUM MAJUS*

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INTRODUCTION

THE investigations on *Tropaeolum* reported upon in this paper were begun in 1929 and continued to 1935. The work is incomplete in many respects, especially as regards the inheritance of anthocyanin flower colours, but the present author is unable to continue the investigations and the results obtained seem of sufficient interest to warrant publication.

So far, very little has been published upon the genetics of *Tropaeolum*. Rasmuson (1918, 1920) reported upon the inheritance of plastid colour in flowers and leaf colours, but his data are meagre. Correns in 1920 investigated the genetics of variegation and light green leaves. During the years 1911-18 a considerable amount of work, principally on the inheritance of sex and doubleness, was carried on by the late Mr Bateson at this Institution. The results of this investigation were never published, and the material has since been lost. Some of Bateson's data on the inheritance of ivy leaf and variegation have been included in the present paper.

MORPHOLOGICAL FACTORS

Three factors affecting the external morphology have been isolated, as follows:

b produces dwarf bushy habit.

i produces smaller leaves with deeper lobes; petals narrow with coarsely serrated edge.

d gives double flowers, the petals, stamens and carpels all being more numerous.

The factor B

The bush or dwarf habit is characterized by short compact growth with an absence of long trailing shoots. Dwarf habit is completely recessive to the tall straggling type. The factors **B** and **b** gave the following segregation:

TABLE I

Bb selfed

Family	Origin	B	b
3/31	2 ⁴ /30 selfed	18	4
13/31	15 ⁹ /30 "	13	8
14/31	16 ⁶ /30 "	38	15
6/34	6 ¹ /33 "	26	12
9/34	9 ⁷ /33 "	5	3
10/34	9 ⁸ /33 "	10	4
41/34	26 ¹¹ /33 "	141	43
	Total	251	89
	Calculated	255.25	84.75

Bb × bb

1/31	1 ¹ /30 × 2 ⁴ /30	17	8
2/31	1 ² /30 × 2 ⁴ /30	7	8
11/31	13 ⁶ /30 × 16 ⁶ /30	4	4
12/31	13 ¹¹ /30 × 15 ⁹ /30	18	17
	Total	46	37
	Calculated	40.5	40.5

The factor I

The ivy-leaved type is completely recessive to normal leaf. In **i** plants the leaves are more lobed, while the petals are narrow with a coarsely serrated distal edge. Ivy leaf is frequently accompanied by a considerable amount of male sterility, this being especially the case when it is combined with other recessive factors. **I** and **i** gave the following segregation:

TABLE II

Ii selfed

Family	Origin	I	i
3/31	2 ⁴ /30	15	7
14/31	16 ⁶ /30	46	7
1/32	2 ⁹ /31	4	1
10 families from Bateson's records		162	51
Total		227	66
Calculated		219.25	73.25
One family from Bateson's records, Ii × ii		21	19
Calculated		20	20

The factor D

The factor **d** produces doubles of the now well-known "Golden Gleam" type. The flowers tend to be double throughout, having more numerous petals, stamens and frequently carpels than in the normal. These doubles produce plenty of good pollen and are quite fertile. **d** is completely recessive and segregates in a normal single-factor ratio, as follows:

TABLE III

Dd selfed

Family	Origin	D	d
41/34	26 ¹¹ /33	148	45
		26	12
	Total	174	57
	Calculated	173.75	57.25

FACTORS AFFECTING LEAF COLOUR

Three factors for leaf colour are known, two of these, **X** and **Y**, being complementary, as follows:

vv plants have variegated leaves.

xxyy plants have yellow-green leaves.

Xxyy, **XXyy**, **xxYy** or **xxYY** have medium green leaves.

XxYy, **XXYy**, **XxYY** or **XXYY** have dark green leaves.

The factor V

The data for the inheritance of this factor are taken entirely from Bateson's work. Variegation varies considerably in amount, the leaves sometimes showing a fine mottling of white, sometimes large white patches occupying a considerable portion of the leaf surface. Variegated leaf is a simple recessive to whole coloured leaf.

TABLE IV

Vv selfed

	V	v
Total of 30 families	847	273
Calculated	840	280

The two factors X and Y

Three shades of leaf colour occur which are easily distinguishable in scoring—yellow-green, medium green and dark green. As previously shown by Rasmuson (1920), two complementary factors are concerned

with the inheritance of these shades. When both recessives are present, the leaves are light yellow green (**xxyy**). When one or other dominant is present the leaves are medium green, and when both dominants are present the leaves are dark green. Medium green of the four different possible genotypes cannot be distinguished phenotypically. It should, however, be possible to obtain dark greens from crossing the two types of medium green together. In family 11/33, in which all types should occur, several medium green plants were crossed by pollen from a single medium green. Of these one cross, $11^{35}/33 \times 11^3/33$, gave dark greens in the next generation, showing that the two types of medium green, due to different factors, actually do occur.

The data in Table 5 from crosses between different leaf colours give a close agreement with expectation.

The fact that the two types of medium green cannot be distinguished gives modifications of the expected ratio. On selfing a plant of the constitution **XxYy** the unusual ratio 9 : 6 : 1 occurs in the next-generation (see tables).

FLOWER COLOURS

The flower colours may be divided into two types:

- (1) Plastid colours, i.e. yellow and primrose;
- (2) Anthocyanin sap colours, i.e. pink, scarlet, etc. The plastid colours are inherited independently from the anthocyanin colours.

(1) *Plastid colours*

Rasmuson (1918) reported a single-factor difference between dark yellow and light yellow (yellow and primrose), but his data are very meagre. In the present investigations some of the earlier families gave 3 : 1 ratios as if a single factor difference was involved, but later families gave ratios which diverged widely and made it necessary to consider some other scheme to account for the facts. Two complementary factors, **P** and **Q**, appear to be involved, as in the case of the leaf colours. When both one or other of the factors are recessive the flowers are primrose. When both are dominant the flowers are yellow. Thus **pq**, **Pq**, and **pQ** are all primrose, while **PQ** flowers are yellow.

The segregation of yellow and primrose depends upon the constitution of the primrose plant used in the original cross, i.e. whether recessive for both factors or for one only. The F_2 families 3, 6, 7/31 and 29/34 gave a close approximation to 3 : 1 yellow to primrose, the primrose used in these cases being presumably **ppQQ** or **PPqq**. Three other

TABLE V

F₂ from (medium green × light green) selfed (Xxyy or xxYy selfed)

Family	Origin	Medium green	Light green
8/31	9 ¹¹ /30 selfed	35	21
23/31	14 ¹⁶ /30 "	6	6
23/32	33 ¹ /31 "	27	10
29/34	19 ³ /33 "	49	21
40/34	25 ²³ /33 "	50	16
Total		167	74
Calculated		180.75	60.25

F₂ from (dark green × medium green) selfed (XxYY or XXYy selfed)

3/31	2 ¹ /30 selfed	16	6
6/31	5 ¹ /30 "	40	11
13/33	10 ²⁵ /32 "	54	21
14/33	15 ¹⁸ /32 "	23	4
31/34	22 ¹⁸ /33 "	73	20
34/34	23 ² /33 "	51	20
41/34	26 ¹¹ /33 "	150	50
Total		407	132
Calculated		404.25	134.75

F₂ from (dark green × light green) selfed (XxYy selfed)

Family	Origin	Dark green	Medium green	Light green
10/31	12 ⁵ /30 selfed	17	17	2
13/31	15 ⁹ /30 "	12	8	1
14/31	16 ⁶ /30 "	37	13	3
11/33	10 ⁸ /32 "	36	24	6
Total		102	62	12
Calculated		99	66	11

Heterozygous dark green × medium green (XxYY × XxYy or xxYY)

Family	Origin	Dark green	Medium green
1/31	1 ¹ /30 × 2 ⁴ /30	13	12
2/31	1 ² /30 × 2 ⁴ /30	6	9
21/31	23 ⁸ /30 × 12 ⁵ /30	24	29
2/32	2 ⁶ /31 × 2 ³ /31	3	3
23/33	16 ¹⁸ /32 × 21 ² /32	19	22
28/34	17 ¹⁰ /33 × 23 ³⁵ /33	16	10
32/34	22 ²² /33 × 16 ²⁰ /33	27	19
Total		108	104
Calculated		106	106

Heterozygous dark green × light green (XxYy × xxyy)

Family	Origin	Dark green	Medium green	Light green
9/31	12 ² /30 × 10 ¹⁷ /30	9	15	6
11/31	13 ⁹ /30 × 16 ⁹ /30	1	6	1
12/31	13 ¹¹ /30 × 15 ⁹ /30	10	18	7
Total		20	39	14
Calculated		18.25	36.50	18.25

Heterozygous green, type A × heterozygous green, type B (Xxyy × xxYy)

15/34	11 ²⁵ /33 × 11 ²¹ /33	8	23	6
Calculated		9.25	18.50	9.25

F_2 families, 6/31 and 31 and 40/34, gave F_2 ratios which do not differ significantly from 9 : 7, the primrose being presumably **ppqq**. Two families, 10/31 and 23/31, have 23 to 12 and 24 to 13 yellows and primroses respectively. These figures might represent either a 3 : 1 or 9 : 7 ratio, as they do not differ significantly from either; they have therefore been omitted from the data.

The following are the data for F_2 segregations:

TABLE VI
PPQq or PpQQ selfed

Family	Origin	Yellow	Primrose
3/31	2 ⁴ /30 selfed	19	3
6/31	5 ⁵ /30 "	39	12
7/31	7 ¹³ /30 "	19	6
29/34	19 ⁹ /33 "	54	14
	Total	131	35
	Calculated	124.5	41.5

PpQq selfed

18/32	21 ⁴⁸ /31	7	6
6/34	6 ¹ /33	16	22
31/34	22 ¹⁸ /33	49	40
40/34	25 ²⁸ /33	34	26
	Total	106	94
	Calculated	112.5	87.5

The families resulting from back-crosses give widely different ratios, all of which are, however, explicable in the two-factor basis. The following are the data for back-crosses:

TABLE VII

Family	Cross	Yellow	Primrose
21/31	23 ⁸ /30 × 12 ⁵ /30 (PpQq × ppQQ)	26	27
	Calculated on 1 : 1 ratio	26.50	26.50
32/34	22 ²⁸ /33 × 16 ³⁰ /33 (PPQq × ppQq)	34	11
	Calculated on 3 : 1 ratio	33.75	11.25
39/34	25 ¹⁸ /33 × 17 ⁸ /33 (PpQq × ppqq)	19	58
	Calculated on 1 : 3 ratio	19.25	57.75

The back-cross in family 32/34 gives results consistent with selfing plants from the same F_1 family. Family 40/34, F_2 from yellow × primrose, gave a 9 : 7 ratio, showing the primrose to have been recessive for both factors. The back-cross of the same F_1 which was selfed to obtain 40/34 resulted in a ratio of 1 : 3, which was to be expected on back-crossing a plant heterozygous for two factors. These results therefore support the two-factor hypothesis.

(2) *Anthocyanin colours*

The presence or absence of anthocyanin colour in the petals is determined by a single factor, the presence of anthocyanin being dominant to its absence. The factor **A** gave the segregation:

TABLE VIII

Cross		A	a
Aa selfed (20 families)		642	213
	Calculated	641.25	213
Aa × aa (9 families)		142	142
	Calculated	142	142

The inheritance of anthocyanin colours is complicated by the interaction of other genes, giving a wide range of colours which has not yet been fully analysed. Chemical tests made by Miss Scott-Moncrieff of this Institution have shown that two types of anthocyanin occur, a glycoside of pelargonin and one of cyanidin. The main colours found are pink, deep red, chocolate and scarlet. Pink and the scarlets contain pelargonidin derivatives, while deep red and chocolate contain cyanidin derivatives. These colours differ in appearance on primrose or yellow plastid grounds, and with other factors such as that for dark green leaves. A recessive factor (or factors) produces anthocyanin in the styles, reduces petal size and intensifies anthocyanin colours. This factor is sometimes difficult to score, and has not yet been fully analysed. It gave the following segregation:

TABLE IX

Family	Origin	S	s
3/31	24/30 selfed	18	2
6/31	5 ⁶ /30 "	31	3
10/31	15 ⁶ /30 "	22	2
14/31	16 ⁶ /30 "	37	8
11/33	10 ⁹ /32 "	38	8
13/33	10 ²⁵ /32 "	44	11
14/33	16 ⁶ /32 "	21	4
30/34	20 ⁸ /32 "	35	5
31/34	22 ¹⁸ /32 "	70	20
34/34	23 ⁹ /33 "	56	9
41/34	26 ¹¹ /33 "	128	34
	Total, observed	500	106
	Calculated on 3 : 1	454.5	151.5

It is possible that the smaller number of recessives is due to mistakes in scoring, since the tingeing on the style is sometimes very slight, and might be overlooked.

There is considerable evidence to show that deep red is the expression, on plants with medium green leaves, of the factor which produces choco-

late flowers on plants with dark green leaves. Both types of flower contain cyanidin derivatives. Chocolate flowers never occur in combination with medium green leaves. In family 13/33, the result of selfing a chocolate plant 10²⁵/32 segregating for dark green and medium green leaves, the medium green leaved plants had in every case deep red flowers, the ratio being:

TABLE X

Family	Origin	Chocolate flower, dark leaves	Deep red flower, medium green leaves
13/33	10 ²⁵ /32 selfed	41	14

So far, the following factors which affect flower colour have been isolated:

a, absence of anthocyanin colour in the petals.

r, pink, opposed to deep red or chocolate.

c, pink or chocolate, opposed to scarlet.

s, tinged style, intensifies colour.

l, whole colour as opposed to blotch (localized colour).

The factors C and R

C and **R** may be carried by plants having no anthocyanin in the petals, and do not show their effect until **a** is present. In every case it was found that the primroses or yellows used at first carried either **C**, **R** or both. Three types of primrose have since been isolated:

- (1) Primrose **ccrr**, on crossing with pink gives a pink F_1 .
- (2) " **ccRR**, " " deep red F_1 .
- (3) " **CCRR**, " " scarlet F_1 .

The primrose of type 1 on crossing to pink gave in the F_2 :

Table XI

Family	Origin	Pink	Primrose
37/34	24 ¹ /33 selfed	25	4
38/34	24 ⁴ /33 "	53	25
	Total	78	29
	Calculated	80.25	26.75

The pinks in this family were redder than the normal pinks, probably owing to the action of a minor modifier. Pink \times primrose carrying **R** gave in the F_1 deep red, and in the F_2 and back-cross:

TABLE XII

Family	Origin	R	r
5/31	4 ⁴ /30 selfed	19	3
	Calculated	16.5	5.5
20/31	23 ¹² /30 \times 12 ⁵ /30	12	5
	Calculated	8.5	8.5

Pink \times scarlet or yellow carrying **C** and **R** gave a scarlet F_1 which on selfing and back-crossing gave:

TABLE XIII

Family	Origin	Scarlet	Deep red	Pink
40/34	25 ²⁸ /33, CcRr , selfed	43	7	2
	Calculated on 12 : 3 : 1	39	9.25	3.25
39/34	25 ¹⁸ /33, CcRr \times 17 ⁸ /33, ccrr	43	15	20
	Calculated on 2 : 1 : 1	39	19.5	19.5

These ratios give a very close approximation to expectation on the assumption that **C** acts independently of **R** and can itself change pink to scarlet. The scarlets of the constitution **CR** and **Cr** are indistinguishable, giving the observed modifications of the two-factor self- and back-crossed ratios.

Deep red and scarlet

The following ratios were observed in crosses between scarlet, **CR** and deep red, **cR**. **R** was in some cases carried by primrose and also by chocolate. Only plants carrying **R** are included in the data.

TABLE XIV

Family	Origin	Phenotype of F_1 parents	CR	cR
3/31	2 ⁴ /30 selfed	Scarlet \times primrose	17	3
6/31	5 ⁸ /30 "	" "	24	8
7/31	7 ¹³ /30 "	Scarlet \times chocolate	15	10
10/31	12 ⁹ /30 "	Yellow (MN) \times chocolate	19	7
		Total	75	28
		Calculated	77.25	25.75

Back-crosses

Family	Origin	CR	cR
1/31	1 ¹ /30 \times 2 ⁴ /30	7	11
17/31	22 ⁸ /30 \times 7 ¹³ /30	17	28
18/31	22 ⁸ /30 \times 12 ⁹ /30	21	18
21/31	23 ⁸ /30 \times 12 ⁹ /30	22	30
14/34	11 ³³ /33 \times 11 ³¹ /33	14	17
	Total	81	104

TWO-FACTOR RATIOS

TABLE XV

(**BBii** \times **bbII**) selfed

Family	Origin	BI	Bi	bI	bi
3/31	2 ⁴ /30 selfed	11	7	4	0
4/31	16 ⁸ /30 "	33	5	13	2
1/32	2 ⁴ /31 "	3	0	1	1
	Total	47	12	18	3
	Calculated	45	15	15	5

Table XV (cont.)
(**BBAA** × **bbaa**) *selfed*

Family	Origin	BA	Ba	bA	ba
3/31	24/30 <i>selfed</i>	16	2	4	0
14/31	16 ¹ /30 „	32	6	13	1
	Total	48	8	17	1
	Calculated	41.6	13.9	13.9	4.6

(**bbAA** × **BBaa**) *selfed*

41/34	26 ¹¹ /33	115	38	37	6
	Calculated	110.25	36.75	36.75	12.25

(**AADD** × **aadd**) *selfed*

Family	Origin	AD	Ad	aD	ad
41/34	26 ¹¹ /33	118	34	33	11
	Calculated	110.25	36.75	36.75	12.25

Bushy × *Double*, **BBdd** × **bbDD**

Family	Origin	BD	Bd	bD	bd
41/34	26 ¹¹ /33	115	37	36	8
	Calculated	110.25	36.75	36.75	12.25

DISCUSSION

The factors **B**, **I**, **D**, **V** and **A** segregate in close agreement with the expected monofactorial ratios. Data for **B**, **I** and **A** were obtained from back-crossing as well as selfing F_1 's, while the numbers from **D** and **V** were from selfing only. Ratios for **S** give too few recessives for a normal monofactorial segregation, but this may be due to the difficulty of detecting colour in the recessives.

The two pairs of complementary factors, **X**, **Y** and **P**, **Q** differ slightly in their relations. In the former case, each of the dominant genes concerned has some effect in the absence of the other, giving medium green leaves instead of light green; and both factors together intensify this effect by giving dark green leaves. With the plastid genes, neither dominant has any effect alone. The ratios for both pairs of genes agree closely with expectation.

In the absence of modifiers the anthocyanin factor **A** produces pink flower colour. **R** turns pink into deep red, and it appears that **X** and **Y** also interact with **R**, both leaf-colour genes being necessary to convert deep red to chocolate. **C** is probably quite independent of **R** in its action. It modifies deep red to scarlet, and probably gives scarlet flowers also in the absence of **R**, though it remains to obtain scarlet flowers from crossing pink (**ccrr**) with a plant carrying the scarlet but not the red factor (**CCrr**), to prove this independence.

It has been tentatively suggested by Miss Scott-Moncrieff that **R** is a factor for the production of cyanidin (deep red and chocolate) instead of the pelargonidin of the **rr** plants; and that **C** inhibits **R** and intensifies pelargonidin coloration. **C** is epistatic to **R**.

Other minor modifiers of petal colour apparently occur, e.g. an intensifier of pink anthocyanin colour.

From the double factor ratios given, there is no evidence of linkage between the pairs of genes **B** and **I**, **B** and **D**, **A** and **D**; but **B** and **A** may be linked.

SUMMARY

1. The inheritance of 13 genes in *Tropaeolum majus* has been studied.
2. These genes include:
Three controlling morphological characters (**B**, **I**, **D**).
Three controlling leaf colour (**V**, **X**, **Y**).
Seven controlling flower colour, (1) plastids (**P**, **Q**), (2) anthocyanin (**A**, **C**, **R**, **S**, **L**).
3. There are two pairs of complementary genes, **X**, **Y** and **P**, **Q**.
4. The presence of **A** is necessary for the expression of the other genes for anthocyanin colour in the petals; **C** is epistatic to **R**; and minor modifiers of colour occur.
5. No certain linkage has been found between any of the genes.

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A PROBABLE CASE OF CYTOPLASMIC
INHERITANCE IN MAN: A CRITIQUE
OF LEBER'S DISEASE

By Y. IMAI AND D. MORIWAKI

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HEREDITARY optic nerve atrophy, first described by Leber in 1871, is known as Leber's disease. This malady occurs from generation to generation in the affected pedigree, the mode of inheritance, however, being complicated with particular behaviour. It may appear at any time during life from about 4 or 5 to 50 years or even later, but mostly about 20.

The distribution of the disease is world-wide, and so far over 200 pedigrees have been recorded, of which forty-five are from Japan. The pedigree reported by Yang (1923) from a Chinese family, differing as it does from the cases usually met with, is simple in inheritance, being clearly transmitted as autosomal monogenic dominant. Since this apparently exceptional case is due to an autosomal gene that causes Leber's disease with similar diagnosis, it will be excluded from the discussion that follows.

Leber's disease has been described with pedigree charts, and its inheritance discussed, especially by Hormuth, Nettleship, Fleischer & Josenhans, Drexel, Waardenburg, Kawakami, Kitashima, Bell, Hogben, Komai, etc. (cf. Waardenburg, 1932; Komai, 1934). This disease, which is believed to be transmitted generally as recessive, occurs more frequently in the male. With certain particular modes of inheritance it becomes complicated. Our present knowledge of its genetics may be summed up as follows:

(1) The disease has been known simply as sex-linked recessive. Davenport (1930), who in his views went further, wrote:

"Moreover, the trait of hereditary optic atrophy is sometimes induced by a gene that is not located in the sex-chromosome."

Hogben (1932), who made a statistical examination of the subject based on Bell's monograph (1931), however, reached the following conclusion:

"There are two forms of Leber's disease, both genetical types being found among Europeans and probably both among Japanese, though the

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majority of examples of the recessive sex-linked form are European and the majority of the Japanese cases are of the type which is apparently determined by two autosomal dominant genes."

(2) Although the disease is thought to be recessive, it sometimes appears as dominant. According to Kawakami (1926), the nature of incomplete dominance is:

"Sie (die Erbanlage von dieser Krankheit) ist nicht immer rezessiv im weiblichen Geschlecht, sondern häufig dominant, oder sagen wir besser, die Anlage ist dominanzwechselnd im weiblichen Geschlecht."

Meyer-Riemsloh (1925) and Waardenburg (1932) also reached the same conclusion.

(3) One of the most peculiar points of inheritance is what is known as Lossen's law. Lossen pointed out in 1877 that, in haemophilia, the affected male does not transmit his disease to his direct and indirect descendants. This was later denied. The law that he discovered, however, may be recognized in Leber's disease, although a few cases that have been described contradict it (cf. Davenport, 1930). Regarding the mechanism underlying the operation of Lossen's law in this case, Kawakami (1926) wrote:

"Die Lossensche Regel kann man wie folgt beschreiben: Es gäbe eine Eizelle, deren X-Chromosom die Erbanlage dieser Krankheit hat.

1. Wenn die Eizelle den ein X-Chromosom enthaltenden Spermatozoon konzipiert, d. h. Mädchen geboren wird, verschwindet die Erbanlage nicht.

2. Wenn die Eizelle den kein X-Chromosom enthaltenden Spermatozoon konzipiert, d. h. Knabe geboren wird, verschwindet die Erbanlage.

So sei das Y-Chromosom die einzige Ursache von Verschwinden der Erbanlage."

He (Kawakami, 1932) even went further and postulated mutation of the morbid gene to its normal allele through influence of the Y-chromosome.

Komai (1934), on the other hand, stated as follows:

"The trait is autosomal, not sex-linked, and it shows dominance in males always, in females exceptionally. The sperms loaded with the gene of this disease are less viable than the sperms without it; but the viability of the egg is not impaired by this gene. Because of the sex-limited tendency, the disease appears practically in men alone in Western pedigrees. In Japanese pedigrees, however, this tendency is less marked and more women are affected. Next, because of the peculiarity in sperms, the

sperms loaded with the morbid gene take part in fertilization very rarely, hence Lossen's law."

In contrast to these investigators, Waardenburg (1932) states:

"Wenn nun die obengenannte Wahrnehmung der Lossenschen Regel zutrifft... dann sieht man sich genötigt anzunehmen, dass die defekten Eizellen der Töchter befallener Männer (die ja alle Konduktor sind) gerade das Umgekehrte darbieten von den defekten Eizellen der Töchter von Konduktoren, da die ersteren nämlich zur Elimination, die letzteren zur selektiven Befruchtung veranlagt sind. Die wahrscheinliche Lösung derartiger Kontroversen könnte meines Erachtens nur darin gesucht werden, dass man eine Wechselbeziehung zwischen Keimplasma und Zytoplasma annimmt. Jeder Mensch bekommt sein Zytoplasma mütterlicherseits (matroclin). Die weiblichen Konduktoren, die von einer mütterlichen Konduktor stammen haben ein Zytoplasma bekommen, das diese Wechselbeziehungen schon erlitten hat, die weiblichen Konduktoren, die von einem befallenen Vater stammen bekommen ein defektes Chromosom in einem diesbezüglich unberührten Zytoplasma."

(4) Kitashima (1930) verified Meyer-Riemsloh's views (1925) by finding that almost all normal females of the affected pedigrees are carriers of the disease—a fact sometimes called Kitashima's law. This is one of the peculiar features regarding the inheritance of Leber's disease that is difficult to explain. To do so, Meyer-Riemsloh (1925) and Waardenburg (1932) suggested selective fertilization, which, however, remains as mere speculation.

Obviously, the genetics of the disease are not yet completely solved: the theories hitherto advanced fail to explain the facts fully. In going through the literature of human genetics¹ we were struck with the complicated way in which Leber's disease is transmitted, and, as an aid to its understanding, have formulated a working hypothesis. Our opinion, as will be explained presently, seems to throw some light on this mystifying problem.

Waardenburg's opinion harmonizes with ours to a certain extent. Our opinion is that the cause of Leber's disease lies in abnormal cytoplasm. In fertilization, the spermatozoon seems scarcely to contribute any available cytoplasm to the ovum, although it carries some differentiated cytoplasm in its head and neck, from which it follows that the inheritance of cytoplasm may be maternal. Abnormal cytoplasm may cause Leber's disease through stimulation of the sex hormones, with

¹ We are under obligations to Dr R. Kawakami, who kindly allowed us the perusal of some of his literature on the subject.

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different effects in the female from that in the male. It is, therefore, a case of sex-controlled inheritance. Under this condition, the disease is transmitted through the female alone, the male not inheriting this disease—Lossen's law. Since the females of the affected pedigree have abnormal cytoplasm, they are always carriers of the disease—Kitashima's law.

The frequency distribution of the affected females as compared with that of the affected males differs with different races. Kawakami's table (1932) gives 8.4 per cent. for the German, 20.6 per cent. for the French, 21.6 per cent. for the English, and 70.0 per cent. for the Japanese, the average being 25.6 per cent. How are we to explain these differences? We make the tentative suggestion that this difference may lie in the particular property of the cytoplasm. The affected pedigrees seem to fall into two groups, in one of which, with cytoplasm less susceptible to the disease, it appears frequently in the male, but rarely in the female, as the result of differences in the effects of the male and female hormones. Inheritance of the disease almost accords with the simple sex-linked scheme. In the other group, the cytoplasm has a greater sensibility for the female hormone, hence more females are affected than would be expected from ordinary sex-linked inheritance. The majority of the affected European pedigrees seem to contain the former cytoplasm, and the others the latter, while in the Japanese pedigrees the case is reversed. For this reason the affected females are proportionally high in the Japanese. The chance of attack in members of the pedigrees carrying abnormal cytoplasm is thus roughly restricted in its frequency, though it differs to some extent with different pedigrees or races. The disease, probably owing to its close connexion with sex hormones, attacks individuals oftener during the ages from 15 to 30, though the frequencies describe somewhat different curves with different races (cf. Kawakami, 1926; Kitashima, 1930). Seeing that the disease sometimes appears late in life, it is only natural to suppose that some at least are not affected during their lifetime and are hence recorded as normals. There is difficulty in accepting the general assumption that all individuals with full disease heritage (in our hypothesis, defective cytoplasm) should be affected by the disease. Our view is that the female of the affected pedigree may be either healthy or diseased, the disease appearing in her progeny almost irrespective¹ of her own traits, so that the dominance

¹ Davenport's table (1930) shows 63 per cent. affected sons and 7.9 per cent. affected daughters from the conductor mothers and normal fathers, and 73 and 17 per cent. respectively from the affected mothers and normal fathers, thus showing some difference. The difference, however, is not conclusive, because the data from which the percentages of the latter mating were calculated are small in number.

and recessiveness of the disease and their reversal phenomenon seem to lose their meaning in the present case.

CONCLUSION

The cause of Leber's disease probably lies in abnormal cytoplasm, inheritance being therefore maternal (Lossen's law). The abnormal cytoplasm continues in the egg of the affected pedigrees from generation to generation, so that all females are carriers of the disease (Kitashima's law). The disease, in its attack, is stimulated by a sex hormone, especially that secreted by the male. The frequency distribution of the disease therefore differs in the two sexes. In some European, and in the majority of Japanese pedigrees, the proportion of females affected is higher than in the ordinary case, due probably to the higher sensibility of the cytoplasm carried by the particular pedigrees to stimulation of the female hormone, so that transmission of the disease is a case of sex-controlled inheritance. The Chinese case reported by Yang has a different cause, being due to an autosomal dominant gene.

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GENO- AND PLASMOTYPES OF VARIEGATED PELARGONIUMS

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(With Plates III-VI and Seventeen Text-figures)

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INTRODUCTION

SINCE Baur's classical experiments (1909) with pelargoniums these plants have served as excellent material for experiment to a number of geneticists (Bateson, 1919, 1921; Noack, 1922, 1924, 1925, 1930; Chittenden, 1925, 1927; Roth, 1927), who utilized them to investigate plastid inheritance and the mechanism of chimeras by extending Baur's theories. The garden pelargoniums contain several species or varieties, though they mostly belong to *Pelargonium zonale*. A few decades ago some chlorophyll periclinal stocks were introduced into Japan, soon after which their cultivation became a vogue. Breeders supplied many new forms by means of hybridization and bud variation. When the fashion was at its height the most prized stock fetched more than 1300 Yen. We have now about 110 named stocks of various chlorophyll types.

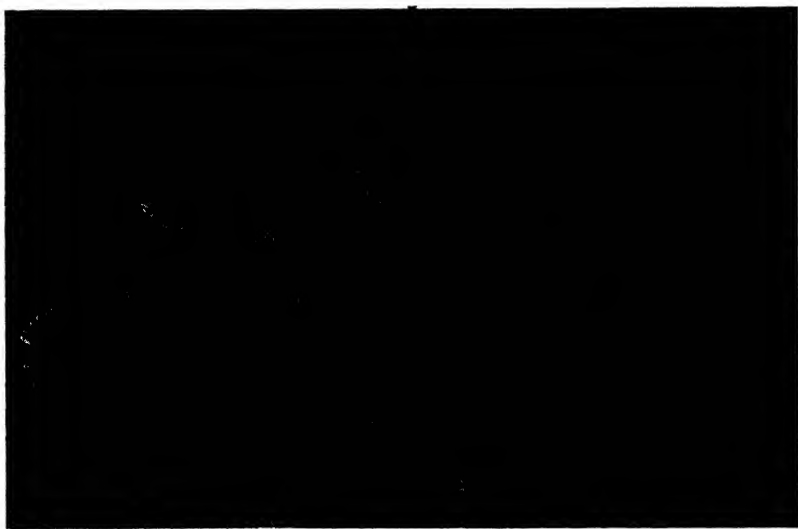
BLACK AND *AUREA* GENOTYPES

The zonal pelargoniums, as the name implies, have usually dark purple circles in their leaves (Pl. III, fig. 1), which is due to the anthocyanin contained in the palisade cells underlying the upper epidermis. The other mesophyll of the leaves is green without anthocyanin, but when the plants are exposed to low temperatures the leaves turn red because of

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the development of pigment in the epidermis and mesophyll. Besides this, we have some stocks of which the leaves are entirely dark purple (Pl. III, fig. 2). The dark purple leaves, which will be referred to as black in this paper, are so beautiful that they are cultivated as ornamental stocks. These black stocks are stunted in growth with small leaves. The anthocyanin is present in the epidermis and mesophyll, especially in the latter, and due to the coexistence of purplish red and green pigments the leaves assume a dark purple colour. In the stems and petioles, the anthocyanin is mostly contained in the cortex parenchyma.

On selfing black stocks I obtained 60 black and 21 green seedlings. The black seedlings were of stunted growth compared with the sister



Text-fig. 1. Four normal and three black seedlings.

green seedlings (Text-fig. 1). Since segregation occurred in accordance with a monogenic ratio, the parental black stocks are regarded as heterozygous for a dominant gene, black. The heterozygous black stocks, when reciprocally back-crossed to green, resulted in 57 black and 64 green seedlings, segregation occurring in almost equal proportion as expected. The black seedlings become apparent when they send out the cotyledons, which are black.

The yellowish green, or *aurea* form (Pl. III, fig. 3), is known to be heterozygous for a bleaching gene (Baur, 1907; Noack, 1924; Roth, 1927). The homozygous *aurea* is yellow and, being a zygotic sublethal, dies

either at the embryonic stage or soon after germination. My breeding experiments led me to expect this, only a few yellows having germinated. Selfing *aurea* stocks resulted in 129 green, 266 *aurea* and 3 non-viable yellow seedlings. The germination percentage, which in normal greens was 51.5, dropped to 37.0 for selfed *aurea*, indicating non-germination of the majority of the yellow seeds. The *aurea* stocks, when back-crossed with green in reciprocal ways, produced 97 green and 95 *aurea* seedlings as expected.

Kingetsu, one of the *aurea* stocks, is an *aurea*-over-green periclinal (Pl. III, fig. 4). The constitution of the stock is

Ectohistogen	...	<i>aurea</i>
Mesohistogen	...	<i>aurea</i>
Endohistogen	...	green

Since the guard cells of the epidermis have yellowish green plastids, the stock is an *aurea* periclinal with green core. The origin of the *aurea*-over-green stock not being clearly known, I cannot draw any conclusions as to the origin of the periclinal, though it seems probable that it was derived through somatic gene mutation of *aurea* to green. Although I have yet neither observed nor heard of any somatic mutation of this kind, the body constitution of Kingetsu seems to imply this probability. A single gene mutation of *aurea* to green might result in an *aurea*-over-green periclinal.

PLASMOTYPES OF PLASTIDS

The protoplasm itself plays an important role as idioplasm, for the hereditary diversity of which the term plasmatype will be used. So far as the present paper is concerned, the plasmatype of plastids is referred to. In our pelargoniums, chlorophyll variation, excepting *aurea*, seems due to variant plasmatypes of plastids.

The various plasmatypes of plastids in pelargoniums are mainly concerned with colour, the crumpling of tissues, and mutability. The normal plastids that contain ample chlorophyll have a green colour. Roughly speaking, the variant colours are yellow (Pl. III, fig. 7), cream (Pl. III, fig. 8), and white (Pl. III, fig. 9), although they exhibit some minor variations. The yellow or cream colours, which are rather intense in the young leaves, fade away to whitish when old. The so-called white is also creamish in young leaves. Albinotic tissues frequently accompany the crumpling of leaves owing to poor development of the affected cells. Sometimes the crumpling is very marked, especially in some white

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leaves. The plastids, when they have constant plasmotypes, propagate true to their own forms, but when they are mutable occasionally change into different plasmotypes. The change of plastids is either automutation or exomutation. The mutation rate is roughly determined by the property of the plasmotypes, although environmental differences cause fluctuation to a certain extent. The ordinary yellow plastids are generally constant, while cream and some white plastids are mutable. Although the green



Text-fig. 2. White-over-green, Matsuenishiki, sporting to narrowly fringed periclinal.

plastids themselves always seem constant, they may mutate through the effect of a stimulant gene, hence the exomutation of plastids.

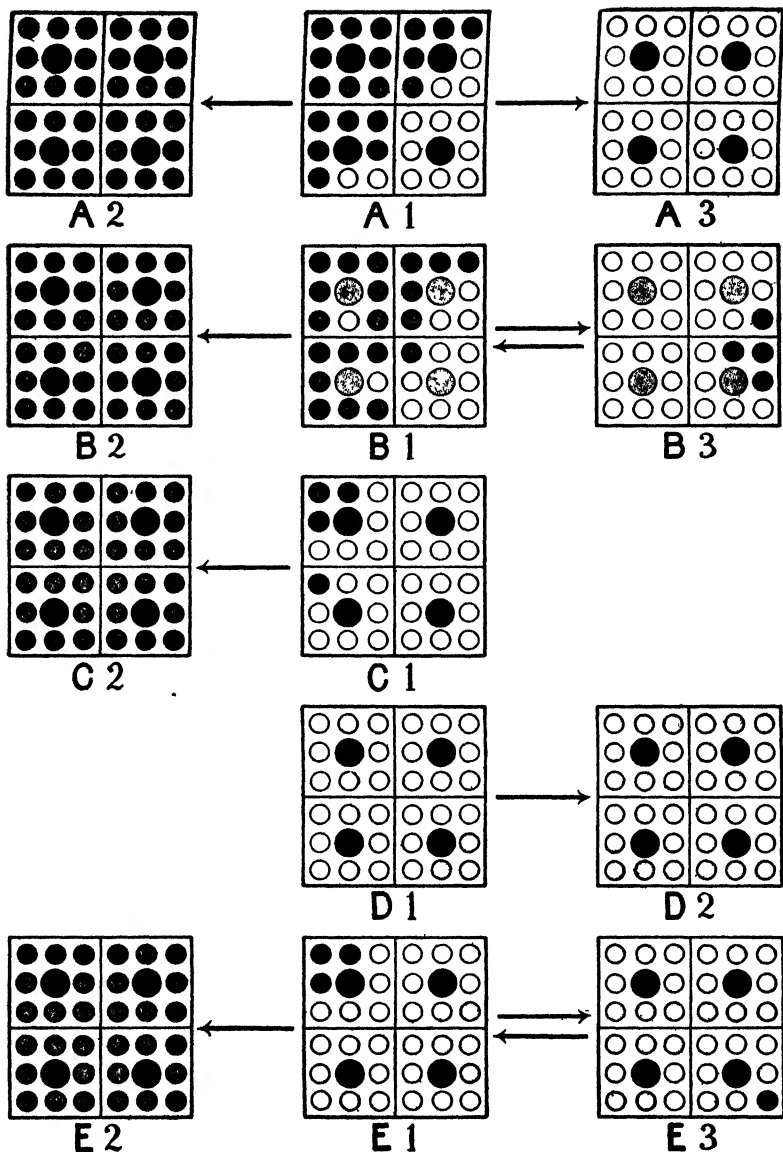
On Fujinishiki, which is a yellow-skinned periclinal, a sport (Pl. VI, fig. 20) with narrow yellow-fringed leaves appeared, the form having been propagated by cuttings. Anatomical observations show that the new form is also periclinal, but with small albinotic cells. The change is possibly due to spontaneous plastid mutation from ordinary yellow to new yellow that lowers the development of the affected cells. Similar sports are occasionally observed in other periclinal stocks (Text-fig. 2).

VARIEGATED FORMS

Two types of variegated forms are found in our pelargoniums, the one due to a mixture of chlorophyll-deficient plastids that were, in the majority of cases, introduced by hybridization or had appeared by spontaneous mutation in infrequent cases and propagated in sporophytic cells, the other being due to recurrent plastid mutation as the effect of a stimulant gene (Pl. III, fig. 5). In the former type of variegation, sorting out of abnormal plastids results in albinotic patches in the course of plant growth. Variegation of this kind is therefore apt to sort out purely into green or albinotic (Text-fig. 3 A), as the result of which bud variation is produced. We have several commercial stocks with variegated leaves of this kind. Maintenance of the form, however, is rather difficult, because the variegation is apt to sort out. The bud variations, which are frequently observable on variegated stocks, are green, albinotic, periclinal (Pl. IV, fig. 16), and reversal (Pl. III, fig. 10). The green sport has no value for ornamental purposes, while the yellow sport is non-viable by itself. The ultimate products of the variegated form are therefore periclinal and reversal, the last-named being generally less esteemed from the ornamental standpoint.

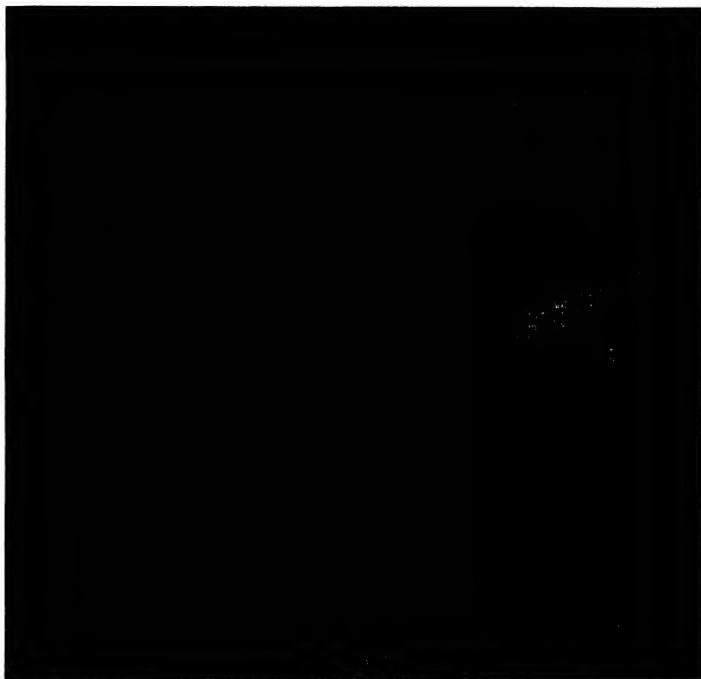
Theoretically the variegated stocks due to mixed mutant plastids should give rise to green, variegated, and albinotic seedlings in irregular proportions according to the degree of variegation. On selfing Unjôni-shiki, a variegated stock, I obtained 56 green and 12 variegated seedlings. In my experiments, as will be seen later, very few albinotic seedlings were produced. As expected, the green sport gave green seedlings only, 52 in all. The origin of the periclinal forms with chlorophyll-deficient histogens is due to secondary production of the variegated forms through somatic segregation.

Another kind of variegation due to recurrent exomutation of plastids from green to albinotic is met with in a white-ticked form, Koshinohomare (Pl. III, fig. 5 and Pl. IV, fig. 17), which is said to have sported from a green stock, Alphonse Riccard. White variegation invariably occurs in the green tissues, the degree to which it appears being somewhat variable. Sometimes the white parts become distributed into large sectors of leaves. The white cells, when they occupy a growing point, grow into a periclinal sport of white-over-variegated, and even into a pure white sport. The white tissue is tinged with cream, especially when the leaves are young. On selfing Koshinohomare, whose fertility is low, I obtained 8 green and 36 variegated offspring. Their identification,



Text-fig. 3. Showing the sorting out of mixed plastids and the propagation of mutant plastids. A 1, variegated tissue due to mixed plastids, sorting out to green and albinotic tissues (A 2 and A 3). Each tissue consists of four cells. The central circles indicate nuclei. The solid nuclei carry the normal genotype, while the dotted one, having a recessive stimulant gene, produces exomutation of plastids. The peripheral small circles around the nucleus represent plastids. The arrows show direction of sporting. B 1, Koshinohomare-variegation, giving all-green and green-ticked white tissues (B 2 and B 3). C 1, albinotic tissue with green ticks, reverting to all-green tissue (C 2). D 1, yellow-ticked Sazanami-variegation, sporting to all-yellow tissue (D 2). E 1, white tissue of Hanamikado containing green and yellow plastids, and its derivative forms, all-green and white-ticked yellow tissues (E 2 and E 3).

however, cannot be made at the seedling stage, because variegation is sometimes not evident until the plant throws out many leaves. The above breeding result is apparently the same as that obtained by selfing variegated stock due to mixed plastids. But, when selfed, the green sport, which occurs at times on the variegated stock Koshinohomare, gave rise to 35 green and 9 variegated offspring, segregating monogenically. This fact indicates the heterozygosity of the green sport, the variegation in the present case being a monogenic recessive to green.



Text-fig. 4. White branch of Koshinohomare. Note green ticks in laminae and stipules.

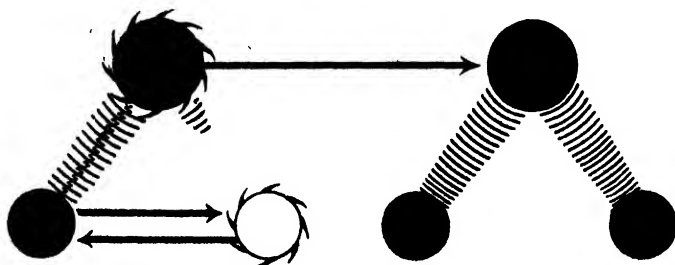
The Koshinohomare variegation is therefore no longer ordinary in its genetic nature. The variegation is maintained in vegetative growth and, provided no further change occurs, the stock is propagated by cuttings without any further selection. We may therefore conclude that the Koshinohomare variegation is due to recurrent plastid mutation controlled by a recessive stimulant gene (cf. Imai, 1934 *a*, 1936). The plastid mutation, therefore, is exomutable. The variegation being recessive to green, the variegated stock, when crossed with green, gave 29 green plants from green ♀ × Koshinohomare ♂, and 7 greens from its reciprocal

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crossing. Because of the fact that white plastids are due to a mutant plasmotype, the cross, green ♀ × white sport of Koshinohomare ♂, gave 20 green and 2 variegated seedlings.

The occurrence of a green sport on Koshinohomare and of green offspring from selfing the stock indicates the mutable nature of the stimulant gene, which induces variegation as the result of recurrent plastid mutation to its dominant green allele. The resultant green is fully constant.

Diagnosis of the white branches of Koshinohomare reveals further genetic complications. It sometimes shows green ticks (Text-fig. 4), though the recurrent frequency is not high. Since the green ticks, which will be fully discussed later, are believed to have originated by plastid



Text-fig. 5. The mechanism of variegation due to stimulant gene, which changes plastids from green to white (exomutation of plastids) and at the same time alters itself to its normal allele (automutation of gene). The larger circles are nuclei and the smaller ones plastids. The solid ripple-like lines show action of nucleus in producing ample chlorophyll pigments in plastids. The zigzag line joining nucleus and plastid indicates the effects of the former in changing the property of the latter. The vanes represent the automutability of the gene and plastid. The arrows show the direction of recurrent mutation. Solid plastids are the green and the blank the albinotic.

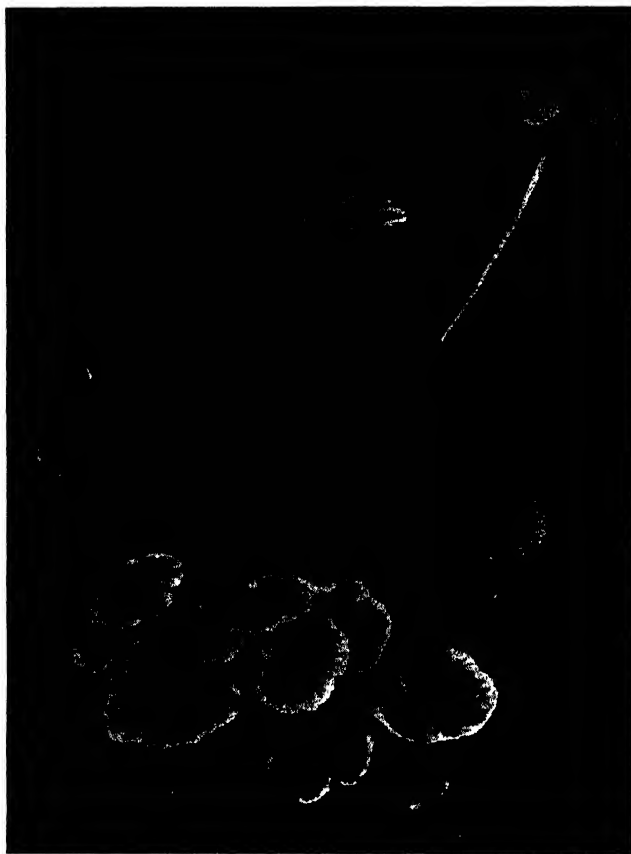
mutation of white to green, recurrent plastid mutation is reversible between green and white, although the frequency differs in two directions. The mechanism of mutation involved in the Koshinohomare variegation is schematized in Text-fig. 5. The relations of geno- and plasmotypes in the Koshinohomare variegation are therefore:

Gene: recessive; the stimulant inducing recurrent plastid mutation from green to white; mutable to normal.

Plastid: exomutable from green to white and automutable from white to green.

As the result of these complex mutations, variegation sorts out differentiated tissues (Text-fig. 3 B). The frequency of exomutation from green to white may differ considerably from that of automutation from white to green, so that it is possible for nearly white sports to occur.

Fujinoyuki (Text-fig. 6), a white-over-green periclinal, produced a sport with yellow-variegated core (Pl. III, fig. 15), the derivative stock being called Chôjuraku (Text-fig. 7). From the latter stock a yellow-variegated sport appeared by throwing off its white skin, the plasmotype being apparently the same as the Koshinohomare variegation, except

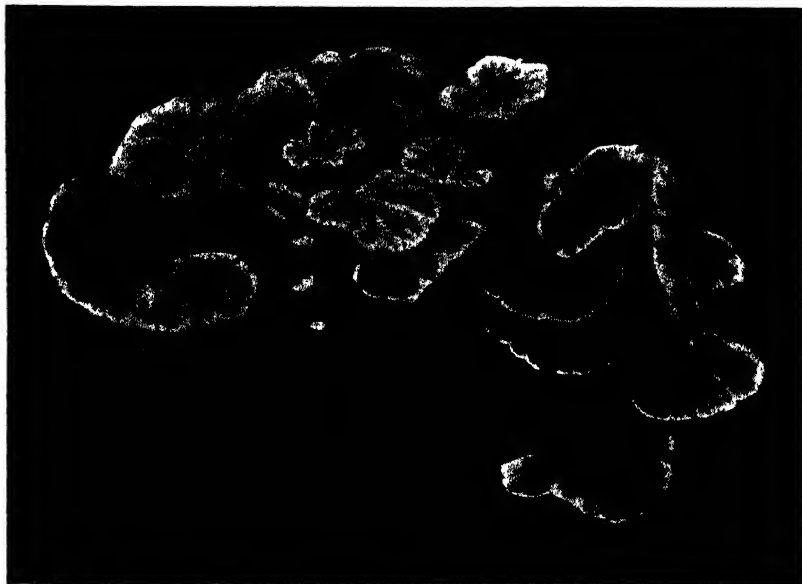


Text-fig. 6. White-over-green, Fujinoyuki, sporting to all-green.

the colour of the albinotic plastids. The core of Chôjuraku, therefore, may be genetically the same as that of Koshinohomare, although there is a difference in the plasmotype of chlorophyll-deficient plastids. Thus, in Koshinohomare, recurrent exomutation occurs from green to white and *vice versa*, while in Chôjuraku it takes place from green to light yellow. The mutated light yellow plastids seem to be constant. The

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leaves of the yellow sport change from greenish yellow to yellowish cream with age. Chôjuraku reverts frequently to Fujinoyuki by gene mutation.



Text-fig. 7. White-over-variegated, Chôjuraku.

PLASTID INHERITANCE

According to Baur's experiments (1909) with the variegated pelargonium, the white tissue contains differentiated, albinotic plastids. The white when selfed gave only white seedlings, and, when it was crossed with green, segregation occurred into green, variegated, and white, but in irregular proportions. Transmission of plastids is thus non-Mendelian. Crossing of green with white gave somewhat different results, according to whether the white is used as the pistillate or the staminate parent. The difference shows that the numerical contribution of plastids through a pollen tube to the zygote is generally lower than through an egg cell. Theoretically, all the F_1 zygotes should be variegated, but in the course of cell generations of their ontogeny some develop as green and albinotic seedlings by the sorting out of one kind of mixed plastids, while others continue the condition as variegated seedlings.

My breeding experiments confirm those of Baur (1909), Noack (1924, 1925), Chittenden (1925), and Roth (1927). So far as my tests go, green

stocks, when selfed or intercrossed, gave only green seedlings. When green was crossed with white, the results from reciprocal crossings were somewhat different, as shown in Tables I and II.

TABLE I

Data from cross, green ♀ × albinotic ♂

Colour of male parent	Cross	Green	Variegated	Albinotic	Total
Yellow	Green × Hinomaru	22	12	0	34
	Green × Yachiyonishiki	64	20	0	84
	Green × Chikyunishiki	10	11	0	21
	Green × Sekainozu	14	8	0	22
	Green × Kinsekai	17	6	0	23
	Green × Kinkeinishiki	45	0	0	45
	Total	172	57	0	229
Cream	Green × Manazuru	262	72	0	334
White	Green × Fujinoyuki	56	2	0	58
	Green × Jindai	9	0	0	9
	Green × Seikaiha	18	0	0	18
	Green × Sazanami	168	0	0	168
	Green × Hanamikado	7	0	0	7
	Total	258	2	0	260
	Totals	692	131	0	823
		84.1 %	15.9 %	0.0 %	

TABLE II

Data from cross, albinotic ♀ × green ♂

Colour of female parent	Cross	Green	Variegated	Albinotic	Total
Yellow	Sekainozu × green	3	3	0	6
	Yachiyonishiki × green	16	1	0	17
	Goshonishiki × green	0	5	1	6
	Hinomaru × green	2	6	1	9
	Total	21	15	2	38
Cream	Manazuru × green	15	18	1	34
	Nishikizuru × green	5	7	0	12
	Total	20	25	1	46
White	Fujinoyuki × green	16	12	0	28
	Jindai × green	7	4	0	11
	Sazanami × green	25	0	0	25
	Seikaiha × green	15	0	0	15
	Kinkan × green	6	0	0	6
	Hanamikado × green	35	0	0	35
	Total	104	16	0	120
	Totals	145	56	3	204
		71.0 %	27.5 %	1.5 %	

Thus, green ♀ × albinotic ♂ gave rise to 84.1 per cent. green, 15.9 per cent. variegated and no albinotic, while its reciprocal mating resulted in 71.0 per cent. green, 27.5 per cent. variegated and 1.5 per cent. albinotic, showing somewhat different proportions of segregation,

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as expected. The percentage of albinotic seedlings differed with different investigators. It was relatively high in Chittenden's experiments, low in Baur's, Roth's, and mine, and nil in Noack's. In my experiments, albinotic or periclinal (white-over-green) branches, when selfed, gave no good seeds. Frequently ovaries grew to produce seeds, but they shrivelled before maturity. Other workers obtained good seeds by the same procedure, although not many. From these results it may be said that the albinotic embryos were partially lethal, it having been possible in my cases for only a few of them to germinate. Although the high mortality of the albinotic embryos is closely connected with chlorophyll deficiency, it is influenced partly by a difference in the conditions in which the experiments were made.

The data of Tables I and II are given separately according to the colour of the parental albinotic tissues. Practically, however, no serious difference was observed in the yellow, cream, and white classes. But close

TABLE III
The germination rate

Cross	Seeds sown	Seedlings	Germination %
Green \times green	985	507	51.5
Green \times albinotic	1397	688	49.3
Albinotic \times green	511	109	21.3

inspection of the data revealed the fact that some crosses gave only green seedlings, and neither variegated nor albinotic. Therefore, some albinotic plastids with very inferior propagation disappear in the variegated embryos during their early ontogeny. Such plants bred all green.

Since the male parent contributes fewer plastids to the zygote than the female parent, more albinotic embryos should be expected from the cross albinotic $\text{♀} \times$ green ♂ than from its reciprocal. In the former cross more heavily variegated zygotes are expected, which have some chance of growing up to albinotic embryos. Since the majority of these albinotic embryos are lethal, the percentage of seed germination from albinotic $\text{♀} \times$ green ♂ should be lower than that from green $\text{♀} \times$ albinotic ♂ . Table III gives their comparison with green control.

The germination rate is 51.5 per cent. in the control. Green $\text{♀} \times$ albinotic ♂ shows nearly the same rate, while from the reciprocal it is less than half.

In the above experiments (cf. Tables I and II) the albinotic parents were white-over-green periclinals or white sports, the two forms, although

they differ in their body constitutions, being the same for germ cells that are derived from mesohistogens.

Periclinal stocks at times throw out green (Text-fig. 6) as well as albinotic sports. Green sports thus produced behave as normal green, giving all green seedlings when selfed.

The reversal stocks (Pl. III, fig. 10), which are green-over-white, having green mesohistogens, should breed true to green. Two reversals were selfed with results as shown in Table IV.

TABLE IV
Data from selfing reversals

Stock	Green	Albinotic	Total
Tanimanoyuki	105	2	107
Takanenishiki	45	0	45
Total	150	2	152

In the offspring of Tanimanoyuki appeared 2 pale yellow seedlings. The spontaneous occurrence of albinos is believed to be due to somatic rearrangement of tissues. Some yellowish endohistogenic cells entered the mesohistogen and contributed to the appearance of albinotic seedlings. The stock Tanimanoyuki is identified with Happy Thought, with which Chittenden (1925) and Roth (1927) made breeding experiments. In my opinion, this form is a green-over-white, but the central parts of the laminae have no green skins and are creamish (Pl. III, fig. 11), so that the form is a *pseudomedioalbinata*, the result probably of toxic affection of the albinotic cores (Imai, 1928, 1934 *b*, 1935 *a*). The border of the creamish parts fades to green. Root cuttings gave pale yellow shoots. Sometimes sports occur in pale yellow as well as in green shoots. On selfing, Chittenden obtained some chlorophyll-deficient forms in otherwise green seedlings. The result seems to be due partly to gene segregation and partly to somatic rearrangement of tissues. Roth obtained some light green seedlings in otherwise greens, probably due to gene segregation. In my stock, except for the appearance of a few pale yellow seedlings, no clear segregation occurred.

COMPLICATIONS DUE TO RECURRENT PLASTID MUTATION

Frequently albinotic tissues of white-over-green periclinal peleroniums have green ticks (Pl. III, fig. 8 and Pl. V, fig. 18). When albinotic sports occur this is rendered much clearer. Chittenden (1925, 1927) particularly called our attention to the significance of the green ticks or lobes in the albinotic tissues. The white or white-over-green branches,

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when selfed, sometimes gave green or variegated seedlings in addition to albinos (Chittenden, 1925, 1927; Roth, 1927). In the case of white-over-green, however, some of them may be attributed to somatic rearrangement of tissues. Since in my experiments selfing of neither white-over-green nor white sports gave any good seeds, I could not test the point. Chittenden observed that the occurrence of green and white seedlings is closely connected with green ticks or lobes. According to my opinion (Imai, 1936), their origin is due to recurrent plastid mutation from albinotic to green.

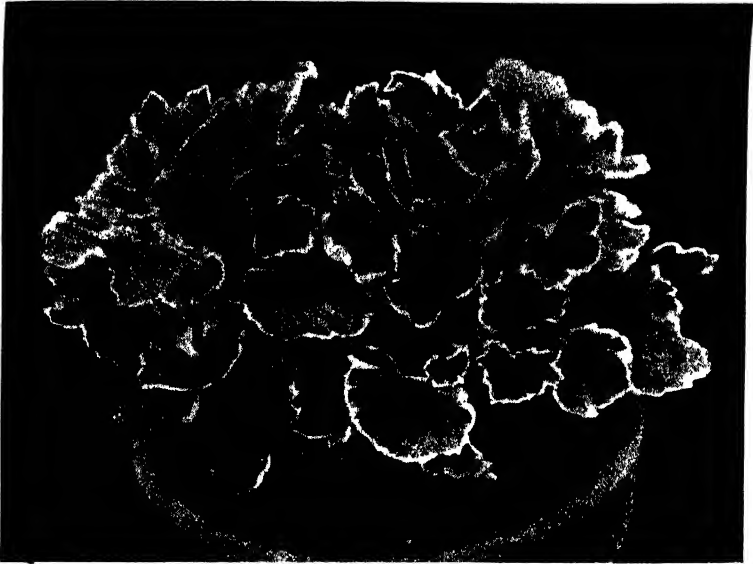
Observations showed that the green ticks occur in the cream and in some of the white tissues, but very seldom in the yellow tissues. Whether they occur or not is due to differences in the plasmotypes of the albinotic plastids, although environment affects their appearance. The green-ticked albinotic has chlorophyll-deficient plastids that are automutable to green, while the non-ticked albinotic are fully constant in their plasmotypes.

The occurrence of green ticks or lobes in leaves and stipules is not concerned with the genotype, but is due to the mutable plasmotype of the albinotic plastid (Text-fig. 3 C). The green parts, however, generally remain as small detached lobes or ticks, indicating that plastid mutation occurs rather late in cell generations of sporophytic ontogeny. If it had occurred early in plant development we should expect green sports that would grow as pure green.

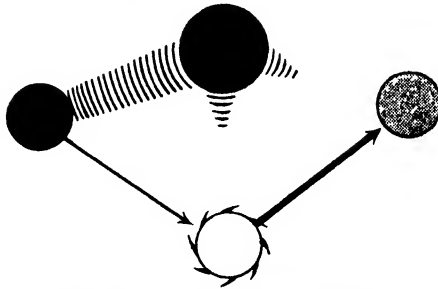
In a white-over-green stock that bears the Japanese name Sazanami (Text-fig. 8) detached yellow lobes (Pl. III, fig. 13) occur instead of green. This stock, which was introduced into this country some time ago, was originally called Golden Brilliantissima, and was investigated by Chittenden (1925). Germination was very poor, but he obtained 7 yellow seedlings by selfing the stock, where whites were also expected. I, however, failed to get any seeds by selfing, although, when foreign pollen of green stocks was applied to the stigmas, many seeds were easily raised as shown in Table II. Further, Chittenden observed yellow-skinned sports, from which 5 yellow seedlings were raised by selfing. The results seem to indicate that the yellow lobes or sports are due to recurrent plastid mutation, the direction of mutation in this case being to constant yellow, but not to green as is usually the case. Text-figs. 9 and 3 D indicate this condition schematically. Sabnis (1932) studied the stock anatomically.

Hanamikado, a white-over-green stock, has a white shrunk skin, which causes the leaves to become much crumpled and serrate in their

margins. The white tissues occasionally contain green ticks, and, though rarely, yellow ticks. Sometimes such yellow parts grow to sports, being



Text-fig. 8. Yellow-lobed white-over-green, Golden Brilliantissima, or Sazanami.

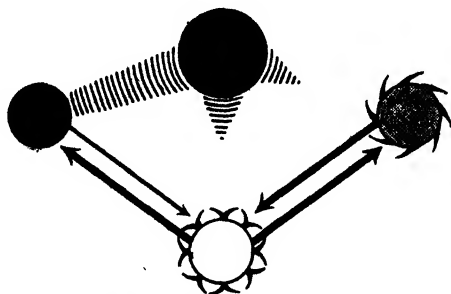


Text-fig. 9. The genetic mechanism of the yellow-lobed white skins of Golden Brilliantissima. The solid plastid is green, the light one yellow, and the blank one white. The ripple-like marks that extend only a short distance out from the centre of the figure indicate the manner in which the plastid character manifests itself, independent of the control of normal nuclear genotype. The thin arrow shows sporadic mutation. For other notes see explanation of Text-fig. 5.

propagated by cuttings under the name Tenshônishiki, which is therefore a yellow-over-green periclinal. The yellow skins, which show little shrinkage, frequently have white ticks (Pl. III, fig. 14) and sports (Pl. VI, fig. 21), the tissues of which are consequently shrunk. From the diagnosis

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it is highly probable that the white ticks appearing in the yellow tissues are due to reversion. Therefore white and yellow plastids can be changed with different frequencies. The white plastid mutates in two directions, green and yellow. The green plastid is constant, whereas the yellow and white are automutable. Text-figs. 10 and 3 E show the mode of mutation schematically.



Text-fig. 10. The genetic mechanism of the white tissue of Hanamikado, changing to green and yellow. The pin-wheel figure with double vanes indicates automutability in two directions.

STRUCTURE OF PERICLINALS

The majority of dicotyledons are usually composed of three histogens (cf. Imai, 1934 *b*, 1935 *a*), and pelargoniums have also this constitution. White-over-green periclinals have white skins and green cores. The so-called skins are developed from ecto- and mesohistogens, and the cores from endohistogens. The ectohistogens develop into epidermis. Generally the epidermal cells of green leaves have a few green plastids, but many plastids are present in guard cells. The chlorophyll characteristic of the epidermis may therefore be seen by examining the colour of the plastids under the microscope, especially in the guard cells. As pointed out by Chittenden (1927), the colour is not always clear enough to enable its difference to be distinguished. In a previous publication (Imai, 1935 *a*) I stated that some periclinals had green plastids in their guard cells, but close examination later showed that observation to be incorrect. Comparative observations showed that the general white-over-green periclinals have albinotic epidermis, though sometimes the evidence for it is obscure. The white-over-green periclinals are therefore white periclinals with green endohistogens. The reason they are not monoheterogeneous, as might be expected from the mechanism by which bud variation occurs (Imai, 1934 *b*, 1935 *b*), will be given later.

Since the reversals, which have a green-over-white arrangement, have green epidermis, they are green periclinals with white cores. In peri-

clinals and reversals, when considered together, the character of the epidermis generally agrees with that of the mesohistogen.

Fubukinomatsu (Pl. V, fig. 19 and Text-fig. 11), or Freak of Nature, bears white leaves with green margins of variable width (Pl. III, fig. 12).



Text-fig. 11. Freak of Nature, or Fubukinomatsu.

Sometimes the green margins appear very faintly as discontinuous dots as a minimum expression, fluctuating to the maximum in forming green laminae with small central whites (Text-fig. 12). The mesophyll is either

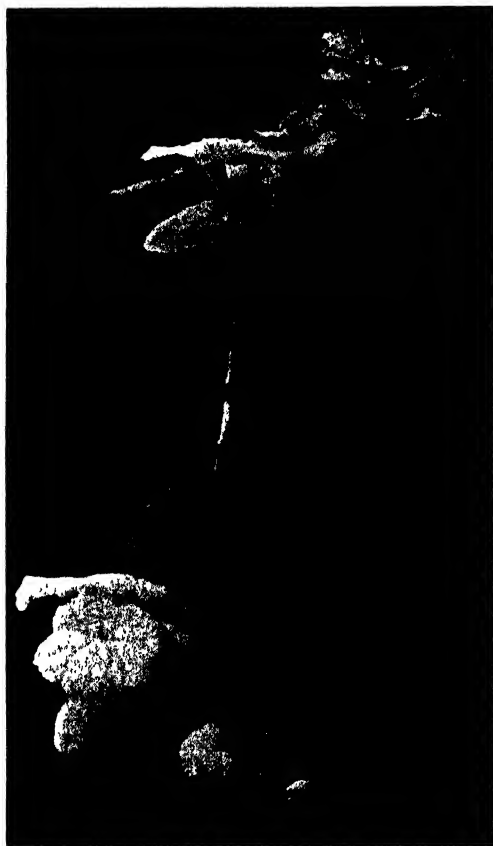


Text-fig. 12. Variation in green margins of leaves in Freak of Nature.

white or green, their borders being quite abrupt. The epidermis, however, is green, even where it lies outside white mesophyll. The stipules are also white with green margins, and of the same constitution as the

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leaves. The stems and petioles are white, but have green epidermis. Green and white sports are frequent (Text-fig. 13). Root cuttings gave white shoots. These white branches frequently have green ticks in the limbs of leaves and in the margins of stipules. Freak of Nature attracted the special attention of Bateson (1919) and Chittenden (1925), while



Text-fig. 13. White and green sports on Freak of Nature.

Noack (1930) fully studied its nature, but, owing to its remarkable peculiarities, its constitution remained a puzzle. Chittenden (1927) redescribed the stock under the section "Peculiar chimaeras", together with some other forms. In my opinion, Freak of Nature is a white periclinal with green ectohistogen, forming green margins to the white leaves, and Noack's breeding results also favour this view. Here we may

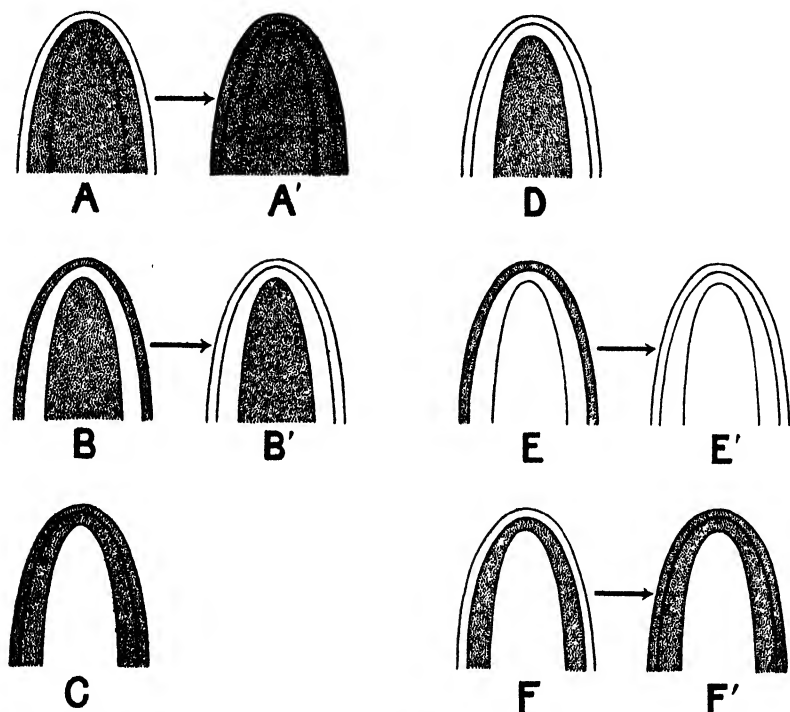
bring into comparison the periclinal *Daphne* (Imai, 1935 *a*), widely cultivated in our gardens, which has, anatomically, a creamish ectohistogen. In this case the ectohistogen is supposed to constitute the marginal mesophyll of the leaves as well as the epidermis. Sometimes we find sports having leaves with broad and creamish margins, besides green and creamish sports. The broad-margined sports have leaves with white-over-green constitution. Since the outer mesophyll underlying the epidermis is albinotic, the green centres of the leaves are of a dull colour. From this fact I concluded that the ectohistogen forms the marginal parts of the mesophyll as well as the epidermis, that the mesohistogen forms the outer mesophyll including the submarginal parts of the mesophyll, and that the endohistogen develops into the innermost central mesophyll. In monocotyledons, which consist of two histogens, the ectohistogen constitutes the marginal parts of the mesophyll as well as the epidermis, the ectohistogen developing into the inner mesophyll. The chlorophyll periclinals, therefore, are *albomarginata* and *medioalbinata* forms.

What has been found in *Daphne* may be applied without any modification to pelargoniums, since both are trihistogenic plants. Anatomical observations of Freak of Nature fully support this view. Chittenden (1925) further obtained the green-over-white sport on Freak of Nature. This form is a common type of reversal, probably having green ecto- and mesohistogens and white endohistogen. Chittenden's variety A (1927) has green leaves with very narrow white margins, their mesophyll being all green. In my view, this stock is a monoheterogeneous periclinal with white ectohistogen. Since albinotic tissue generally develops poorly, the white margins are narrow, while they are broad in the type form of Freak of Nature, in which the green margins oppress the inner white tissues, deforming the leaves. The green leaves with white ectohistogens are *albomarginata* and the white leaves with green ectohistogens are *medioalbinata*.

As stated before, the chlorophyll periclinal pelargoniums generally have ecto- and mesohistogens of the same characters, while only a few forms have different plasmotypes. Freak of Nature and Chittenden's variety A are examples of the latter. The green form is normal and standard, and from it periclinal and reversal forms have been derived through somatic segregation of the albinotic plastids. Therefore a single change in the three histogens is most simple, and should be the first to be found. This expectation, however, contradicts observations of the ordinary white-over-green periclinals. I think that this inconsistency is

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introduced by the instability of somatic tissue arrangement in this trihistogenic plant when the albinotic tissue constitutes one of the two outer histogens. The instability may be due to lack of balance in contact development between the epidermis and the underlying tissue at a growing point. If so, the unstable chlorophyll periclinals should be apt to change into stable forms, as shown in Text-fig. 14, on the formation of



Text-fig. 14. Schematic growing points consisting of three histogens. A, B, and C are monoheterogeneous chlorophyll periclinals, of which A and B, being unstable types, change to A' and B' respectively through replacement of the mesohistogen. C is stable, continuing as a reversal. D, E, and F are diheterogeneous periclinals, of which E and F, being unstable types, transform into E' and F' respectively. D is constant. The dotted portions indicate green histogens and the white ones albinotic histogens.

sports. Theoretically, the chlorophyll periclinals due to somatic segregation of albinotic plastids should, in the majority of cases, be monoheterogeneous, such as A, B, and C in the figure, of which the first-named two, being unstable types, should change into A' and B' respectively. Only periclinal C is constant. A monoheterogeneous periclinal with white ectohistogen thus transforms into homogeneous green, a monoheterogeneous with white mesohistogen into ordinary white-over-

green, while a monoheterogeneous with white endohistogen remains a reversal. The diheterogeneous forms, D, E, and F, become as shown in the diagrams. The stable types, homogeneous green (A'), homogeneous white (E'), ordinary white-over-green (B' and D), and ordinary green-over-white (C and F') are commonly found in our pelargoniums. The white-over-green periclinals produce at times white, green, and reversal sports, and the green-over-white reversals change to green, white, and periclinal. So far as we have examined, these changes occur from stable types to other stables. This fact is proof of the balance theory in chlorophyll periclinals, though it is not found in anthocyanin variegation.

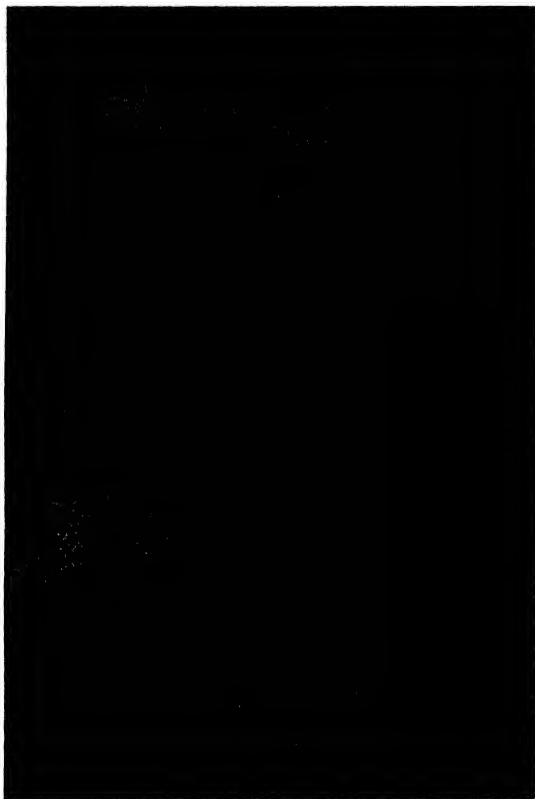
Chittenden's variety A is type A in Text-fig. 14, while Freak of Nature is type E. These forms are unstable types, and their existence seems to be due to the carrying over of the changing period, without accident, at the formation of growing points. After their formation the decisive somatic rearrangement is not followed, so that the stocks maintain their own types without difficulty by vegetative propagation, unless other somatic sports that are frequent in Freak of Nature occur.

Hanamikado has a white shrunken skin, and the marginal white parts of the leaves vary considerably with the different environmental conditions under which the plant is grown. Sometimes the stock puts out green crêpe sports (Text-fig. 15) as well as normal greens and shrunken whites. The leaves of the former have finely serrated margins, frequently reverting to normal green with flat leaves and normal margins. Since, anatomically, the green crêpe is a periclinal with albinotic ectohistogen, the leaves frequently have white fringes to a very faint degree (Pl. III, fig. 6). Owing to very poor development of the albinotic tissues the ectohistogen cannot develop to form the marked fringing. Shrinkage of the leaves is evidently due to the effect of the albinotic ectohistogen.

From what has just been said, it may be concluded that development of the three histogens into plant tissues proceeds as follows: The entire plant body of the pelargonium is composed of three histogens. In leaf ontogeny, the ectohistogen develops into the marginal mesophyll as well as the epidermis, the mesohistogen into the outer mesophyll as well as the submarginal parts of mesophyll, and the endohistogen into the remaining innermost mesophyll (Text-fig. 16). The extent of the contribution of the ectohistogen to leaf ontogeny cannot be traced in the ordinary white-over-green or green-over-white, because the ectohistogen has the same character as the mesohistogen. In some white-over-green periclinals of other dicotyledons the same situation may be expected. From his anatomical views of pelargoniums Noack (1922) concluded

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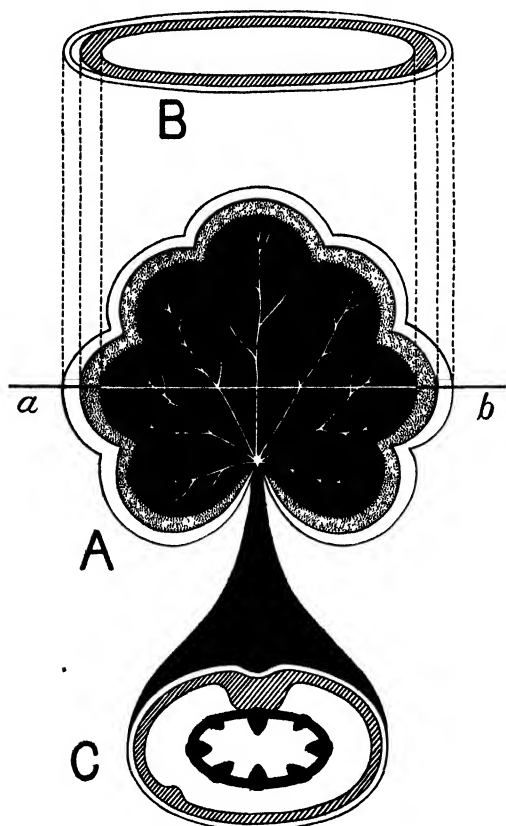
that the epidermis of the leaves is derived from dermatogen, and all the mesophyll from a single histogen underlying the dermatogen. The differentiation of skins and cores in the white-over-green periclinal and its reversal is attributed to somatic segregation occurring at the meristem. His theory provoked strong objections. Chittenden (1927), who did not



Text-fig. 15. Green crêpe sport on Hanamikado. One of the upper leaves has a normal sector.

accept Noack's view, however, used also the term polychlamidius, in which the skin of a periclinal is more than two layers thick. Anatomically, the case is met with in some periclinal pelargoniums—a condition, however, that has no importance genetically, seeing that all the cell layers that constitute a skin are derived from the ecto- and mesohistogens, regardless of their number. The *albomarginata* leaves found in *Veronica* and *Sesamum* were believed to be due to their dihistogenic constitution

(Imai, 1934 *b*). But now there is the possibility that they are equally trihistogenic dicotyledons with colourless ectohistogen, although we hesitate to draw any conclusions. The white-fringed *Fragaria* is also a



Text-fig. 16. Origination of leaf tissues from three histogens. A, the number of histogens contributing various parts of leaves. The black part consists of three histogens, the dotted of two, ecto- and mesohistogens, and the white of one, ectohistogen. B, the cross-section of the leaf at the line *ab*. The outer layer is epidermis and the inner components are mesophyll. The outer white part is developed from ectohistogen, the inner barred part from mesohistogen and the innermost white from endohistogen. C, a cross-section of the petiole. The outer white layer is epidermis, developed from ectohistogen, the inner barred part from mesohistogen, and the innermost from endohistogen, the last-named two parts being cortex and central cylinder.

monoheterogeneous periclinal with white ectohistogen. Even so, I do not think that all dicotyledons are composed of three histogens; such plants as *Polygonum*, *Firmiana*, etc., are considered to be derived from two histogens (Imai, 1934 *a*, 1935 *a*).

In pelargoniums the stipules are composed of three histogens with

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ontogenetical development similar to that of leaves. The transverse sections of stems and petioles of various periclinals and reversals show the extent to which the respective histogens have developed. The conclusion from our observations is that the ectohistogen develops into the epidermis, the mesohistogen into the subepidermal layer or layers of cortex, sometimes some of the inner cortex, and the endohistogen into



Text-fig. 17. Green-over-white, Sangokaku.

the inner cortex including the central cylinder (Text-fig. 16). Under the epidermis of stems and petioles a collenchymatous layer is present that does not contain any chlorophyll pigment, so that the stems and petioles are equally green in the all-green and white-over-green forms, although the latter has whitish stripes in the dorsal parts of the petioles and frequently also in other parts of the petioles and in the stems. Green-over-white reversals have whitish or yellowish stems and petioles (Text-fig. 17) with corresponding green stripes. The cells of such longitudinal stripes are derived from mesohistogens. In other plants, however, the

mesohistogens develop into subepidermal cells several layers thick, so that the stems and petioles of white-over-green are dull green owing to the outer white cortex covering the inner green tissues.

CONCLUSION

Variegated and periclinal pelargoniums offer some very puzzling problems. Chittenden (1927) collected some of these cases, which, however, have become simplified as the result of my investigations. Two forms of variegation were identified, the one due to sorting out of mixed plastids, and the other to recurrent exomutation of plastids induced by a recessive stimulant gene that is mutable itself. Various plasmotypes of plastids differ in their colour, the degree of crumpling of tissues, and mutability. The plasmotypic change of plastids is either automutable or exomutable, while sometimes the plastids transform in two directions. The white-over-green periclinals generally have albinotic epidermis. In the majority of cases unstable chlorophyll periclinal types change into stable types, probably at the growing points. Freak of Nature, Chittenden's variety A, and the green crêpe sport from Hanamikado are periclinals with differentiated ectohistogens, it being green in the first named and albinotic in others. The extent to which the respective three histogens contribute to the ontogeny of the plant body is considered from both anatomical and genetic investigations. Since in leaf formation of the pelargonium the ectohistogen develops into the marginal mesophyll as well as the epidermis, the mesohistogen into the outer and submarginal mesophyll, and the endohistogen into the innermost mesophyll, the chlorophyll periclinals with differentiated ectohistogens bear leaves with margins of a different colour.

I wish to express here my hearty thanks to Prof. K. Miyake for his valuable criticisms with reference to the present investigation, and to the Council of the Hattori-Hokokai for aid received in the form of a grant for prosecuting these studies. The stock material was furnished mainly through the courtesy of Mr S. Tomoda of Tokyo and Mr H. Hamashima of Mumazu, to both of whom I wish to express my thanks for their friendly help.

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EXPLANATION OF PLATES III—VI

PLATE III

The standard and its derivative variant leaves.

- Fig. 1. Normal.
 Fig. 2. Black, Surusumi.
 Fig. 3. Aurea, Ôgon.
 Fig. 4. Aurea-over-green, Kingetsu.
 Fig. 5. Variegated due to recurrent plastid mutation, Koshinohomare.
 Fig. 6. Green with white ectohistogen, sported from Hanamikado.
 Fig. 7. Yellow-over-green, Kinki.
 Fig. 8. Cream-over-green, Konpiranishiki.

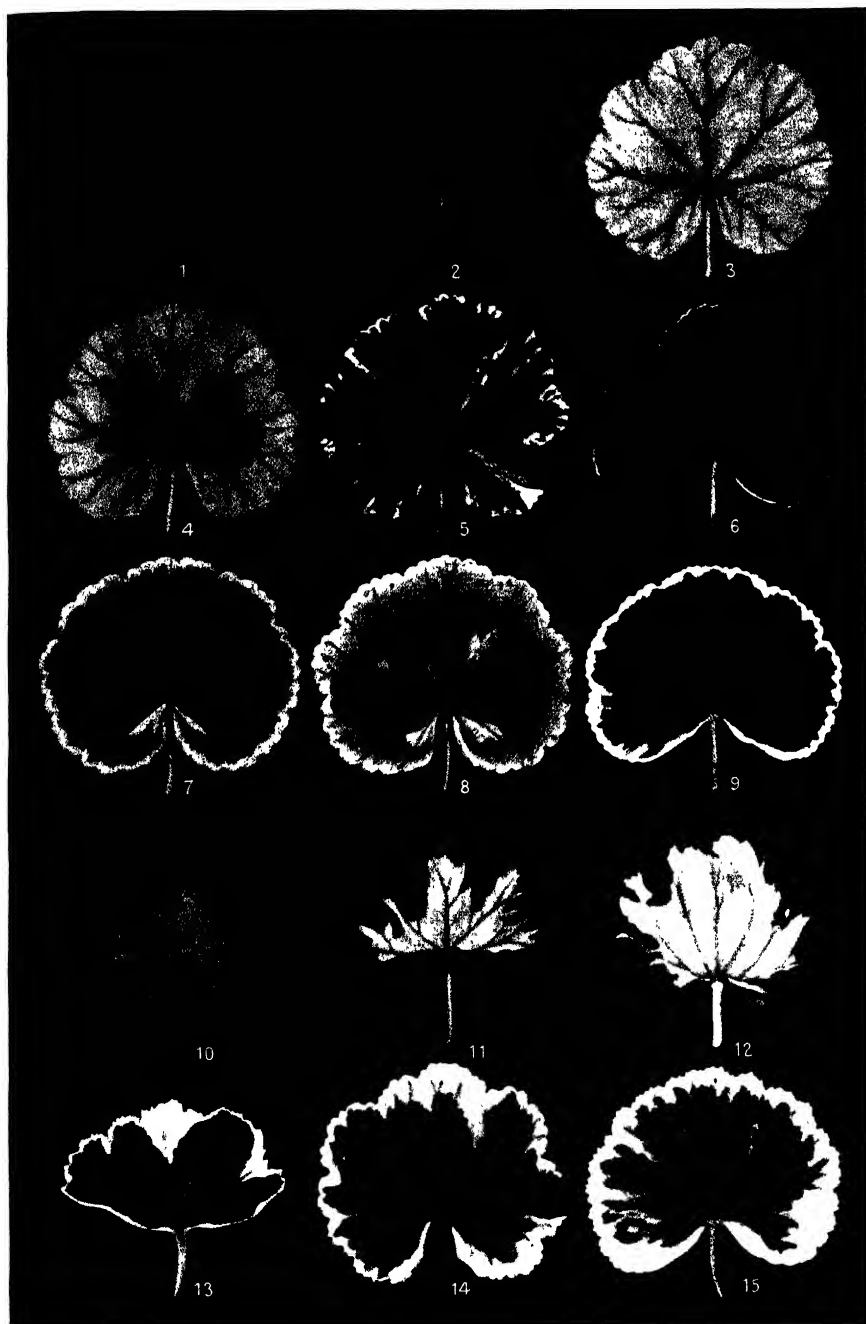




Fig. 16



Fig. 17



Fig. 18



Fig. 19

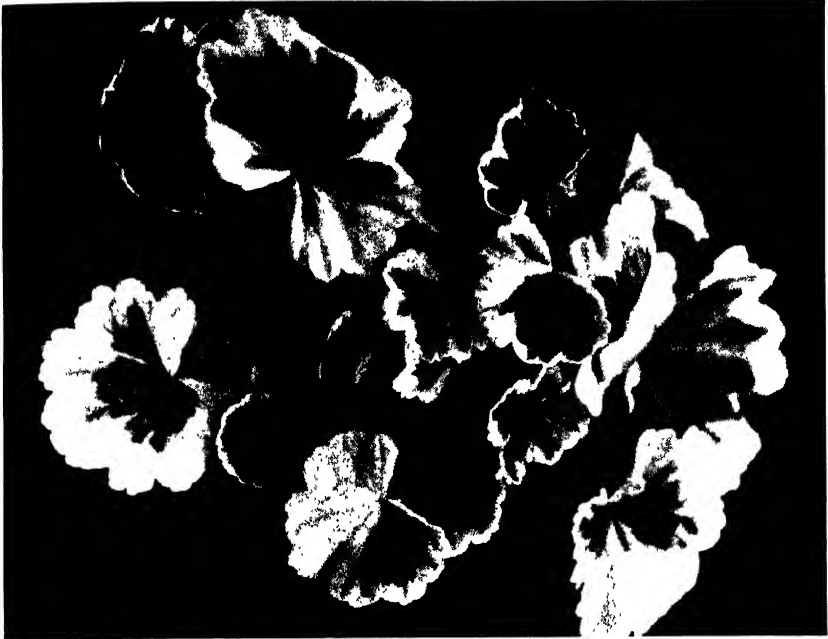


Fig. 21

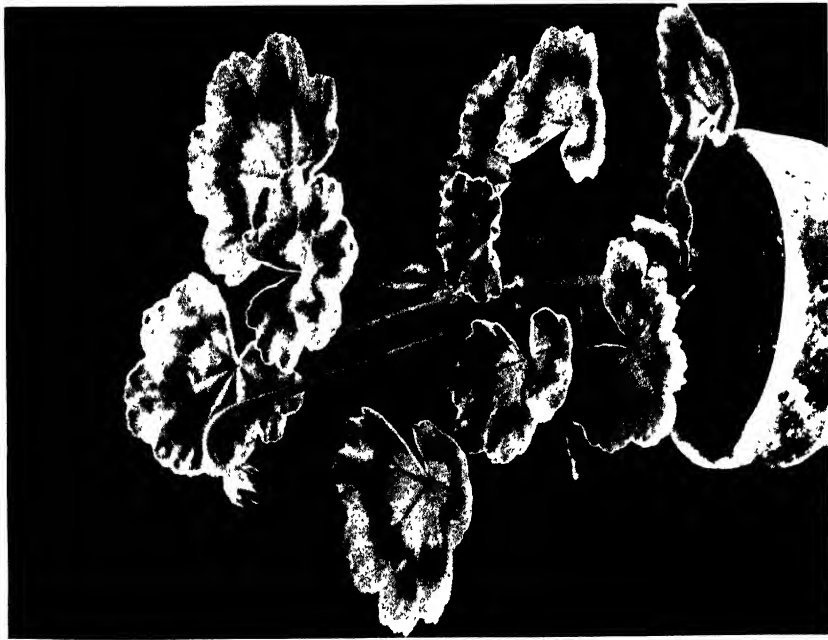


Fig. 20

- Fig. 9. White-over-green, Fujinoyuki.
 Fig. 10. Reversal, Takanenishiki.
 Fig. 11. *Pseudomedioalbinata*, Tanimanoyuki.
 Fig. 12. *Medioalbinata*, Freak of Nature, or Fubukinomatsu.
 Fig. 13. White-over-green with yellow lobes, Golden Brilliantissima, or Sazanami.
 Fig. 14. Yellow-over-green with white lobes, Tenshōnishiki.
 Fig. 15. White-over-variegated, Chōjuraku.

PLATE IV

- Fig. 16. Variegated stock, Shichihōnishiki, sporting to white-over-green periclinal.
 Fig. 17. Variegated, Koshinohomare.

PLATE V

- Fig. 18. Cream-over-green, Shizumatsunishiki. Note green ticks in albinotic skins.
 Fig. 19. *Medioalbinata*, Freak of Nature, or Fubukinomatsu.

PLATE VI

- Fig. 20. Yellow-over-green with narrow fringing, sported from Fujinishiki.
 Fig. 21. Yellow-over-green, Tenshōnishiki, reverting to white-over-green Hanamikado with shrunken leaves.

POSTSCRIPT

Just as I was sending this manuscript to Prof. Punnett, M. Ufer's paper "Erblichkeitsuntersuchungen an 'Freak of Nature'. Ein Beitrag zur Frage der nicht-mendelnden Vererbung chlorophylldefekter Formen von *Pelargonium*" was published in *Z. indukt. Abstamm.- u. VererbLehre*, **74**, 281-98 (1936). Ufer made experiments with Freak of Nature and he concluded that "Freak of Nature ist ebenso wie die albotunikaten Pelargonien als eine Chimäre aus zweierlei genetisch verschiedenen Geweben aufzufassen. Die genetische Verschiedenheit der beiden Gewebe wird durch ungleichen Aufbau der zugehörigen Plasmen bedingt."

CHROMOSOME BEHAVIOUR IN RELATION TO GENETICS OF *AGAVE*

I. SEVEN SPECIES OF FIBRE *AGAVE*

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Amani, Tanga, Tanganyika Territory)

(With Four Text-figures)

IN starting breeding experiments with *Agave* in an endeavour to raise types superior in yield and quality of fibre to those in common cultivation, attention is first being devoted to a study of the species in the section *Rigidae* (Berger) of the subgenus *Euagave* (Baker). Most of the species which have been used as a source of fibre in their home country, Central America and Mexico, belong to this section. Of the thirty-six species included in this group by Berger (1915) thirteen are known to fruit, two rarely fruit, and for the remainder the flowers and fruits are not known. More than half of these species reproduce freely by vegetative means, bulbils or suckers; the manner of reproduction of the remainder is not recorded.

Eleven species of this section are represented in the Amani collection, and the chromosomes of these species are being examined as circumstances permit in an endeavour to form some idea of the origin and relationships of these forms and also in a study of the causes of sterility, partial or complete, which is observed in many of them in East Africa. In this paper the chromosome complements of seven species are studied and the chromosome behaviour of five species at meiosis is described.

MATERIAL AND METHODS

Agave sisalana Perrine was introduced to East Africa from Florida in 1893 and is now widely cultivated in this region (Hindorf, 1925). This species was taken to Florida from Yucatan by Dr Perrine in 1836, and it is from this source that most of the countries cultivating this species, excluding the Central American States, obtained their original material. There is little doubt that this species in the East African territories is a clone.

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A. fourcroydes Lem. is not cultivated in East Africa. A few plants are maintained by H. Tanner, Esq., Amboni, Tanga, to whom I am indebted for my material of this species and also for *A. cantala*, another species which is not grown in this country. These three species are the most important forms cultivated for fibre production.

A. amaniensis, a species of unknown origin recently described under this name by Trelease and Nowell (1933), was found growing in the Amani plantations. It is considered to be allied to the *Zapupe* group of agaves of which *A. Zapupe* Trelease is the type (Trelease, 1909). *A. Lespinassei* Trelease, introduced from Mexico some years ago by the Germans, also belongs to this group. *A. angustifolia* Haw. is not used in fibre production but belongs to the same section of *Agave* as the foregoing, and has many characteristics which may be of use in breeding experiments. This species was also introduced by the Germans.

Root tips have been fixed in a number of fixatives of which 2BE and 2BD (La Cour, 1931) have given the best results. Sections were cut at 24μ and stained by the gentian-violet-iodine method. Pollen mother cell divisions were examined in aceto-carmin and permanent smears (Belling, 1930; La Cour, 1931). Paraffin sections of anthers did not, in most cases, give satisfactory results, fixation being indifferent.

OBSERVATIONS

In Table I are summarized the observations on chromosome numbers and behaviour in the species examined, together with the data from earlier workers on species in this genus.

TABLE I
*Summary of observations on chromosome numbers and behaviour
in Agave species*

	Chromosome number and form				Poly- ploidy	Metaphase configuration	Sterility
	<i>n</i>	<i>2n</i>	Long	Short			
<i>A. amaniensis</i>	30	60	10	50	2x	30 ^{II}	Partial
<i>A. angustifolia</i>	30	60	10	50	2x	30 ^{II}	Partial
<i>A. Lespinassei</i>	—	60	10	50	2x	—	—
<i>A. cantala</i>	90/2	90	15	75	3x	III, II, I	Complete
<i>A. Zapupe</i>	—	ca. 110	20	ca. 90	4x	—	—
<i>A. fourcroydes</i>	ca. 140/2	ca. 140	24	ca. 116	5x	V, IV, III, II, I	Partial
<i>A. sisalana</i>	ca. 138/2	ca. 138	24	ca. 114	5x	V, IV, III, II, I	Partially fertile under certain conditions
<i>A. americana</i>	30	60	10	50	2x	30 ^{II}	— } (McKelvey and Sax, 1933)
<i>A. consociata</i>	30	60	10	50	2x	30 ^{II}	
<i>A. filifera</i>	30	60	10	50	2x	30 ^{II}	
<i>A. virginica</i>	30	60	10	50	2x	30 ^{II}	

N.B. The low numbers recorded by earlier workers (Müller, 1912; Schaffner, 1909; Catalano, 1930) can safely be discredited.

(a) *Somatic chromosomes*

The complements of the first three species ($2n=60$) include ten long chromosomes with subterminal attachment constrictions, two of which show a marked secondary constriction. The remainder of the chromo-

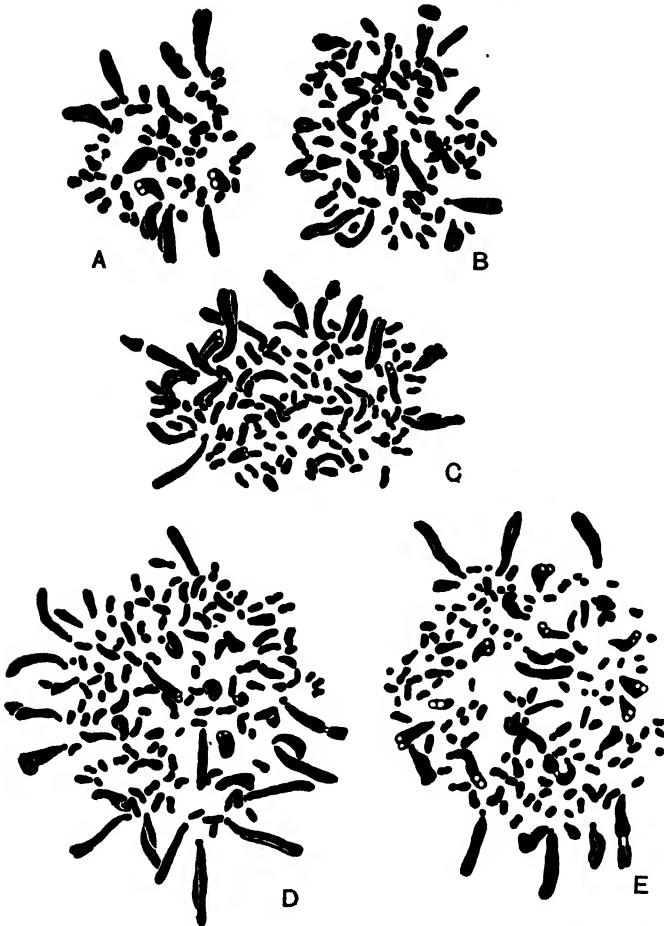


Fig. 1. Mitotic metaphases of *Agave* species. A, *amaniensis* (2x); B, *cantala* (3x); C, *Zapupe* (4x); D, *sisalana* (5x); E, *fourcroydes* (5x). $\times 2250$.

somes are small but differ in size. Fig. 1 A illustrates the chromosomes of *A. amaniensis* at metaphase of mitosis and is typical of these three species. In the other species the small chromosomes are comparable in range of size with those found in the first three. *A. cantala* has fifteen long chromosomes, *Zapupe* twenty, *fourcroydes* and *sisalana* twenty-four. In favourable preparations three of the long chromosomes of *cantala* can

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be seen to have a secondary constriction, four in *Zapuze* and also four in *fourcroydes* and *sisalana*. The complements of these four species are illustrated in Fig. 1 B-E.

(b) Meiosis

In the first meiotic division of the pollen mother cells of *amaniensis* and *angustifolia* thirty bivalents are formed (Fig. 2 A). The pairs of long chromosomes show three to four chiasmata per bivalent with incomplete

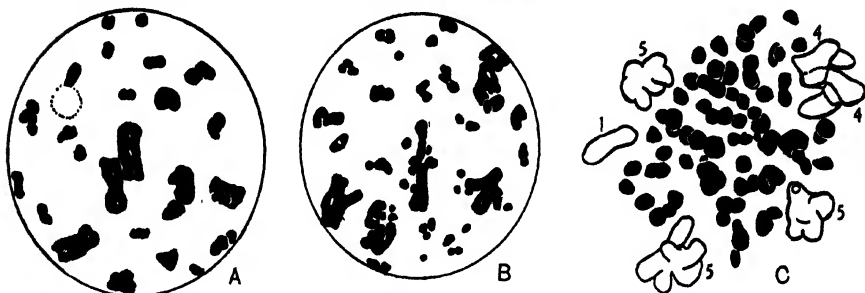


Fig. 2. First division of meiosis. A and B diakinesis. A, *amaniensis* (2x); B, *cantala* (3x); C, polar view metaphase, *sisalana* (5x). A and C $\times 2250$; B $\times 1625$.

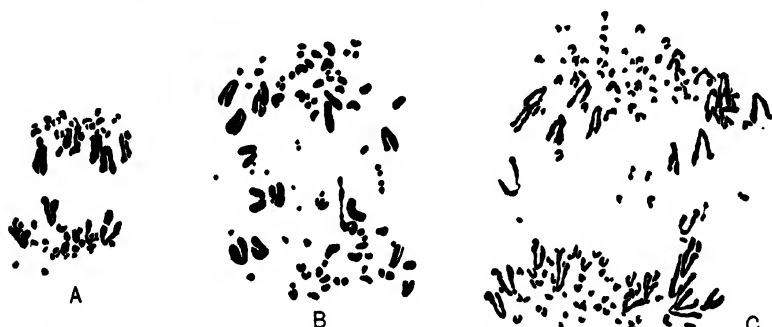


Fig. 3. Anaphase of the first meiotic division showing lagging chromosomes in the polyploids. A, *amaniensis* (2x); B, *cantala* (3x); C, *sisalana* (5x). $\times 1500$.

terminalization. Occasionally chiasma formation in two long chromosomes seems to be hindered by the association of one of the pair with the nucleolus (cf. Darlington, 1935, on *Fritillaria*), but univalent chromosomes have not been observed in these species. An analysis of the number of chiasmata formed between the pairing short chromosomes is difficult but appears to be about 1.3 chiasmata per bivalent on the average. In many cases a single chiasma is formed which is terminalized at an early stage.

Both divisions show, with few exceptions, regular behaviour (Fig. 3 A),

and the resulting pollen grains are uniform in size and very few bad (see section (c)). In the exceptional cases, at first anaphase two chromatids of a pair of long chromosomes fail to separate normally, appearing drawn out across the cell and associated with the formation of a fragment (Fig. 4 A). This behaviour has been shown to follow crossing-over in relatively inverted segments of a pair of chromosomes (cf. Richardson, 1936). The appearance of a chromatid bridge has so far only been observed at first anaphase in *amaniensis* among the diploid species.

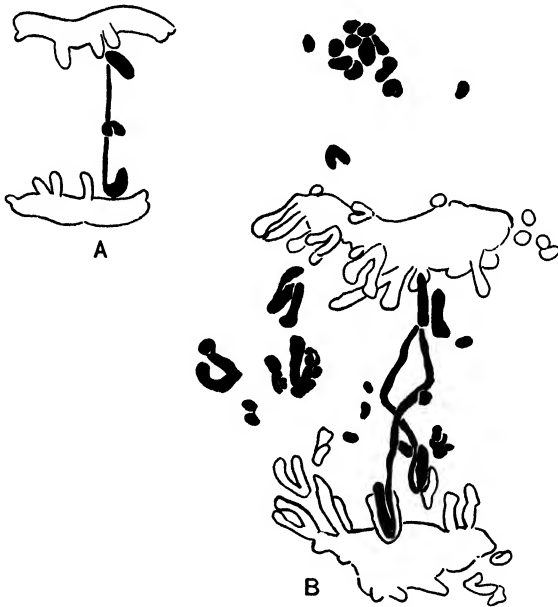


Fig. 4. Chromatid bridges at first anaphase. A, *amaniensis* ($2x$); B, *sisalana* ($5x$). $\times 2250$.

At diakinesis and metaphase of the first division in triploid *cantala*, trivalents, bivalents and univalents are observed among both the long and the short chromosomes (Fig. 2B). An analysis of the configurations shown by the long chromosomes in twenty-three cells gave seven with the possible maximum of five trivalents, nine with four and seven with three, the balance being represented as bivalents and univalents. The proportion of trivalents among the long chromosomes is high, viz. 80 per cent. The proportion of trivalents amongst the short chromosomes, with a chiasma frequency of between 1 and 2 is difficult to estimate. It is certainly lower than among the long chromosomes, probably about 50 per cent., but rather higher than would be expected (cf. Stone and

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Mather on *Hyacinthus*, 1932). The evidence, however, shows that tri-valent formation depends on there being chiasmata available.

At anaphase of both first and second division a varying number of lagging univalents are to be seen (Fig. 3 B). In this species also a number of cells have been seen which show chromatid bridges in long chromosomes. The unequal distribution of chromosomes results in a large proportion of bad pollen, and the seemingly good pollen (tests have shown very poor germination) shows a fairly wide range in size (see section (c)).

Quinquevalents, quadrivalents, trivalents, bivalents and univalents have been observed at the first division in the pentaploid *sisalana* among the long chromosomes. A complete analysis of the configurations has not been made owing to the large number of chromosomes present. Multivalents are also formed among the small chromosomes, but the higher valencies are not as frequent among these smaller units (Fig. 2 C). At first anaphase varying numbers of lagging univalents are observed (Fig. 3 C), and in this species also chromatid bridges, in long chromosomes only, similar to those noted in *amaniensis* and *cantala* have been observed (Fig. 4 B). Although the chromosome distribution is irregular the proportion of bad pollen is not as high as that found in *cantala* (see section (c)).

Flowers of *fourcroydes* at the right stages were scarce, so that only a general idea of the meiotic behaviour of this species has been obtained. The behaviour appears to be similar to that described for *sisalana*, but the proportion of bad pollen is rather higher.

Flowers of *Zapupe* and *Lespinassei* were not available; our plants of these species are still young.

(c) *Pollen*

The proportions of bad pollen and the germination of pollen grains are indicated in Table II.

TABLE II
Proportions of bad pollen and pollen germination

Species	Bad pollen	Germination
	%	%
<i>A. amaniensis</i> (2x)	5	60
<i>A. sisalana</i> (5x)	10	40
<i>A. fourcroydes</i> (5x)	15	—
<i>A. cantala</i> (3x)	40	Less than 1

The first division of the pollen-grain nucleus has not been studied. The thick wall makes fixation and observation difficult. To form some idea of the variation in chromosome numbers in the grains, measurements

of their diameters, which are possibly related to the chromosome number, have been made for comparison. The drawings of pollen grains were made immediately after mounting in aceto-carmin. The results obtained are compared in Table III.

TABLE III
Comparison of the diameters of pollen grains

Species	Mn. μ	S.D.	C.V. $\left(\frac{\text{S.D.}}{\text{Mn.}} \times 100 \right)$	N
<i>A. amaniensis</i> (2x)	76.0	6.8	8.9	60
* <i>A. cantala</i> (3x)	85.4	13.8	16.2	81
<i>A. fourcroydes</i> (5x)	84.2	15.8	18.8	61
<i>A. sisalana</i> (5x)	90.2	11.2	12.4	94

* Excluding all obviously bad pollen.

DISCUSSION

The basic chromosome number for *Agave* appears to be $x=30$. The occurrence of aneuploid forms in the Rigidaceae and other sections of the genus appears to be frequent. The small chromosomes in the high polyploid species are variable in number within the species. The observations of meiosis in the polyploid species indicate that they are autopolyploids. The chromosome behaviour of *sisalana* and *fourcroydes* at meiosis is comparable in many respects with that described for *Tulipa Clusiana* (Newton and Darlington, 1929) and *Ochna serrulata* (Chiarugi and Francini, 1930). *Tulipa Clusiana* was considered to be sterile, but it has fruited this year in the greenhouse at the John Innes Horticultural Institution. *Ochna serrulata* is sexually sterile reproducing apomictically. *Agave fourcroydes* is not completely sterile, whereas *A. sisalana*, sterile when allowed to develop naturally, sets fruit and produces viable seeds under special conditions (Nutman, 1931).

A certain degree of relationship between the species of this group may be inferred from their close resemblance in form and habit of growth, fibre characteristics, and their rather limited distribution in the coastal region of Mexico, Campeche and Yucatan. There is a strong resemblance in the range in size and form of the chromosomes in the species examined. The limited evidence from breeding experiments also indicates a certain degree of relationship. Hybrids have been raised from the cross *angustifolia* (2x) \times *amaniensis* (2x) and reciprocally, and both these species have been successfully crossed with *sisalana* (5x) using *sisalana* pollen. Attempts to cross *amaniensis* (2x) and *cantala* (3x) using *cantala* pollen, however, were unsuccessful. Vidal (1925) reports the success of a cross *fourcroydes* (5x) by *sisalana* (5x). *Angustifolia* (2x) has also been crossed

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reciprocally with *A. ingens* var. *picta* Berger (probably $4x$), a species belonging to another group, *Americanae*, of *Euagave*. Further evidence of affinity or otherwise between these species should be obtained when the F_1 generation comes into flower.

A study of the vegetative characteristics of seedlings of five species of *Agave* indicates that the species are genetically heterozygous, and we have clear evidence of structural hybridity due to chromosome differentiation in the species so far studied. This structural change indicated in some of the long chromosomes of both diploid and polyploid species requires further study.

In the diploid species studied at Amani the failure to set fruits appears to be in part due to the absence of the usual pollinating agent. Artificial pollination results in a much greater set of fruits and seeds, but even then the proportion of ovules which form viable seeds is low. The meiotic divisions of the pollen mother cells have been shown to be relatively effective; the development of the megaspore has not been studied. In the polyploid species a high degree of functional sterility would be expected. The effect of external conditions on fertility in *Agave* has not been studied. The causes of the change from complete sterility to partial fertility in *sisalana* under certain conditions are not yet fully understood, but this change appears to be connected with conditions influencing the activity of an abscission layer which is seen at the junction of the ovary and pedicel and not to any change in the internal reproductive mechanism (Nutman, unpublished).

SUMMARY

1. The somatic chromosome complements of seven species and the meiotic behaviour, in the pollen mother cell divisions, of five of these are described. A basic chromosome number of $x=30$ is inferred.

2. The species most widely cultivated for fibre production are shown to be autopolyploids (*A. sisalana* Perrine ($5x$), *A. fourcroydes* Lem. ($5x$), *A. cantala* Roxbg. ($3x$)).

3. Breeding experiments indicate that the species are genetically heterozygous; structural hybridity and chromosome differentiation is indicated in the meiotic behaviour.

4. A certain degree of relationship between the species in the group Rigidæ, presumed from their similarity in general characteristics and limited distribution, is supported by a comparison of their chromosome complements and by limited evidence from breeding experiments. The diploid species will cross with one another and with polyploid species.

5. Sterility in some of these species is shown to be in part functional and in part influenced by external conditions. The partial sterility of diploid species requires further investigation.

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THE DETERMINATION OF POSITION IN CROSSING-OVER

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(With Six Text-figures)

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I. INTRODUCTION

WITH the discovery of linkage and the formulation of the chromosome theory to explain it, arose the problem of the mechanism of crossing-over. Although *Drosophila* workers originally based their theory of linkage on Janssens's cytological observations, the early work on crossing-over was done by the genetical method. A number of important discoveries resulted, among which were (a) the linear arrangement of the genes (Sturtevant, 1913), (b) the occurrence of interference between the crossing-over of nearby segments (Muller, 1916), (c) the proof that crossing-over occurs in the "four-strand" stage (Bridges, 1916; Bridges & Anderson, 1925, etc.) and, more recently, (d) the demonstration of the independence of the strands crossing-over at different levels, i.e. the absence of chromatid interference (Beadle & Emerson, 1935; Mather, 1933).

The recent demonstration of the chiasmatype theory has made it possible to relate genetical and cytological observations, since on this theory each chiasma represents a point of crossing-over (Darlington, 1935 b; Mather & Lamm, 1935). The two kinds of observation may be compared and combined. Hence, one may utilize the observations on the behaviour of chromosomes in other organisms when considering genetical data from *Drosophila*.

Both genetical and cytological observations have provided evidence

for the belief that the frequency of crossing-over per cytological unit in particular sections of a bivalent is not uniform. The chief sources of evidence on this point are:

(a) The effect of varied environmental conditions, e.g. temperature changes (Plough, 1917, Graubard, 1932), on crossing-over in *Drosophila melanogaster*. Crossing-over near the spindle attachment is affected more than crossing-over in other regions.

(b) Though the cytological and genetical maps of *Drosophila* chromosomes show the same linear order of the genes, the relative distances between the genes in the two types of map are not the same (Dobzhansky, 1930, 1931; Muller & Painter, 1932).

(c) It has been observed cytologically, in a number of organisms having a large range in chromosome size, that while the chiasma frequency (i.e. crossing-over frequency) is proportional to chromosome length for the larger bivalents, this is not true for the short chromosomes (Darlington & Dark, 1932; Darlington, 1932). The short chromosomes have a disproportionately high chiasma frequency in that they always form a chiasma no matter how short they are. This clearly implies some mechanism depending on things other than the chromosome length. If crossing-over were evenly distributed along the length of the chromosomes this would not be the case.

Hence it can be stated that there exists some localized mechanism determining the frequency and distribution of crossing-over in any bivalent. An analysis of existing data should throw light on the position of the determining point or points and on the regional distribution of crossing-over resulting from the mechanism.

II. CROSSING-OVER AND THE SPINDLE ATTACHMENT

A comparison of the cytological and genetical chromosome maps of *Drosophila*, as is shown in Fig. 1, shows an apparent relation between the distribution of crossing-over and the position of the spindle attachment. There is a definite crowding of the loci near the spindle attachment in the genetical map of each chromosome and a corresponding spacing out near the middle of the X-chromosome, with a terminal spindle attachment, and in the middles of the two arms of chromosomes II and III, which have median spindle attachments. Hence it would appear that the crossing-over in any region may be determined by the distance of that region from the spindle attachment. It is clear that the distribution of crossing-over is not determined from the ends of the chromosomes, as in that case the X-chromosome would correspond to

the whole of the second or third chromosomes and not to one arm of them. Fig. 1 shows that this is not the case.

There are other lines of evidence which point to the same conclusion. Beadle (1932) has shown that, in a homozygous III-IV translocation, there is good reason to believe that crossing-over is low in the region of the spindle attachment. In this translocation the bulk of the right arm of chromosome III, from c_a to the right end, has become attached to the small chromosome IV. Beadle found that the regions c_a-s_r and s_r-e^s showed a very marked reduction of crossing-over in the homozygote as compared with the normal fly. The amount of crossing-over between e^s and c_a was unchanged. Hence, moving the bulk of this arm about

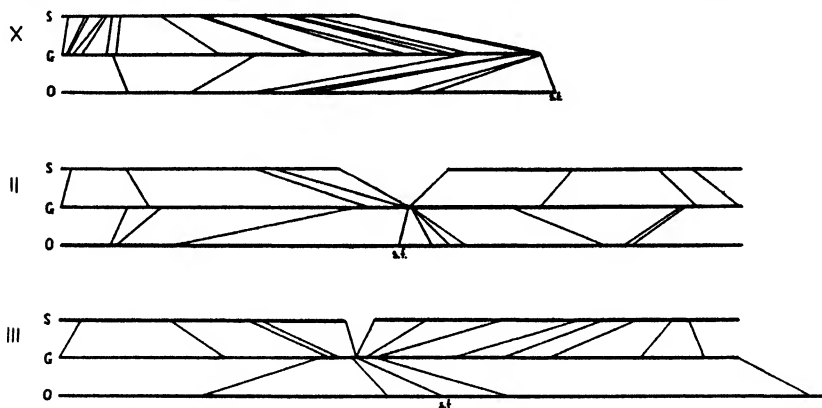


Fig. 1. Diagram to show the relations between genetical (G), mitotic metaphase (O) and salivary gland (S) maps for the three long chromosomes of *Drosophila melanogaster*. Corresponding loci in different maps are joined and the spindle attachment is denoted by $s.f.$ The gaps in the salivary maps of chromosomes II and III are the inert regions whose length cannot be estimated. (Data from Dobzhansky, 1929, 1930; Muller & Painter, 1932; Painter, 1934, 1935; Mackensen, 1935.)

3 genetical units nearer to the spindle fibre resulted in a decrease of crossing-over as far away as e^s . This seems to be convincing evidence that it is the position of the region relative to the spindle attachment, rather than some property of the region itself, which determines the amount of crossing-over in it.

Crossing-over in the two arms of each of chromosomes II and III is independent. This is clearly shown both by the coincidence of crossing-over in regions of the two arms and by the analysis of variance of simultaneous crossing-over in the two arms (Stevens, 1936). This independence has been doubted by both Graubard (1932) and Schweitzer (1935). In the former case the basis for the doubt was the consistent occurrence of coincidence values lower than unity for regions in separate

arms of chromosome II at a number of different temperatures. Stevens (1936) has, however, shown that this result was entirely due to the use of an inconsistent measure of coincidence. With the correct measure, independence is clearly shown. Graubard also tends to this conclusion in a later paper (1934).

Schweitzer's objection is based on an analysis of the relations between the "fraction realized", a measure of coincidence which he invented, and the cytological length of segment exchanged. He maintains that his Fig. 5 shows that all seven regions of chromosome II, as marked in Graubard's (1932) experiments, have practically coincident curves for this relation, irrespective of their position relative to the spindle attachment. A study of his figure leads one to doubt the validity of his conclusion. If the two arms are independent the central regions should show independent crossing-over over smaller distances than the end-regions. Hence the end-regions should, on the whole, have a lower "fraction realized" for any cytological distance than the central regions. This is precisely what Schweitzer's figure appears to show. Regions 1, 2 and 7, which are end-regions, have lower curves than regions 3, 4 and 5, which are central. Region 6 has a slightly lower curve than 3, 4 and 5. Hence the curves in question, far from showing that the spindle attachment does not separate the chromosome into two independent arms, as Schweitzer considered, would appear to point the other way. It cannot be taken as evidence against the present argument.

The evidence available from coincidence studies shows that the two arms of the chromosome with median spindle fibres act independently as regards crossing-over. This is most easily accounted for by supposing that crossing-over is dependent on conditions existing at the spindle attachment. One further consequence of this independence is that for the purpose of our analysis we may now treat the arms of chromosomes II and III as separate and distinct chromosomes.

The variations in crossing-over resulting from changes in the environment, such as temperature (Plough, 1917; Graubard, 1932), X-irradiation (Muller, 1925), are symmetrical about the spindle fibre in chromosomes II and III. The X-chromosome shows a different behaviour which becomes understandable if this chromosome is likened to one arm of the long autosomes. This clearly points to a relationship between the spindle attachment and crossing-over, and emphasizes the unimportance of the ends of the chromosomes in this respect.

This hypothesis of the spindle attachment control of crossing-over, which has been developed from genetical evidence available from *Droso-*

phila experiments, is in good agreement with cytological observation. Darlington (1935 *a*) has shown that in *Fritillaria* species the diplotene separation of the paired chromosomes commences in the regions near the spindle attachment, or centromere, and moves progressively along the chromosomes to the more distal regions. Since separation occurs as a result of the division of each chromosome into two chromatids, which is accompanied by crossing-over and chiasma formation, the agreement of this observation with the hypothesis developed above is excellent.

It is also of some interest that when chiasma formation is localized it is nearly always confined to the spindle attachment region. This seems to imply some relation between crossing-over and the spindle attachment, but the question is complicated by the fact of incomplete pairing of the chromosomes at pachytene.

The evidence reviewed above can leave but little doubt that the region distribution of crossing-over, particularly in *Drosophila melanogaster*, is directly relatable to the spindle attachment. The next question to arise is, of course, what is the nature of this relation? This will now be considered.

III. THE ANALYSIS OF CROSSING-OVER DATA INTO ITS COMPONENTS

The data from multipoint cross-over experiments in *Drosophila* show that single, double, triple and quadruple cross-overs may occur in any chromosome arm. Since, however, triple and higher cross-overs are comparatively rare and so cannot themselves be analysed, we may confine attention to single and double cross-overs. The multiple cross-overs count as doubles for this purpose. The system may be considered as dependent on two variates, viz. (*a*) the cytological distance of the position of the first chiasma, i.e. point of crossing-over, from the spindle attachment, and (*b*) the distance between the position of the first and second chiasmata. The first chiasma is defined as the proximal one in the light of the hypothesis developed in the previous section. This formational seriation of the chiasmata may not be universally true, but the observations quoted above, particularly those of Darlington on *Fritillaria*, would definitely appear to demonstrate its validity in special cases. It will be shown in a later section that this assumption is in fact true in *Drosophila*.

To analyse existing data in terms of these two variables it is necessary to partition the double and multiple cross-overs into their component parts, the one depending on the first chiasma and the other on the second chiasma. Since triple and quadruple chiasma formation is not being considered this simple partition is sufficient for our purpose. It is clearly

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incorrect to make the partition on the basis of the direct genetical results, as the analysis must be in terms of chiasmata, i.e. points of crossing-over, each of which has two of the four strands as cross-overs. So two chiasmata result, on the average, in one no cross-over strand, two single cross-overs and one double cross-over. Hence the frequency of single cross-over strands, for example, is dependent on the frequency of formation of one, two, three, etc., chiasmata. The data can be simply transformed into frequencies of chiasma formation. The method has been developed in an earlier paper (Mather, 1933).

Each chiasma results in two of the four strands showing crossing-over. So the results of n chiasmata in terms of frequencies of strands with various numbers of cross-overs are given by the expansion of

$$(\frac{1}{2} + \frac{1}{2})^n.$$

This is assuming that there is no chromatid interference (see section I). The relations between frequencies of chiasma formation and crossing-over may be shown in a two-way table:

Chiasmata	Crossing-over					
	0	1	2	3	4	...
0	1					
1	0.5	0.5				
2	0.25	0.5	0.25			
3	0.125	0.375	0.375	0.125		
4	0.0625	0.25	0.375	0.25	0.0625	
⋮						etc.

Hence if the frequencies of formation of 0, 1, 2, etc., chiasmata are a, b, c , etc., and the frequencies of recovered strands with 0, 1, 2, etc., cross-overs are α, β, γ , etc., the transformation equations are

$$\alpha = a + 0.5b + 0.25c + 0.125d + 0.0625e \dots,$$

$$\beta = 0.5b + 0.5c + 0.375d + 0.25e \dots,$$

$$\gamma = 0.25c + 0.375d + 0.375e \dots,$$

$$\delta = 0.125d + 0.25e \dots,$$

$$\epsilon = 0.0625e \dots,$$

$$\vdots$$

or alternatively

$$a = \alpha - \beta + \gamma - \delta + \epsilon \dots,$$

$$b = 2\beta - 4\gamma + 6\delta - 8\epsilon \dots,$$

$$c = 4\gamma - 12\delta + 24\epsilon \dots,$$

$$d = 8\delta - 32\epsilon \dots,$$

$$e = 16\epsilon \dots,$$

$$\vdots$$

The crossing-over data used in this analysis have all been transformed into chiasma frequencies by the use of these equations.

The actual data are taken from Anderson & Rhoades (1931), and Morgan (1933) for the X-chromosome, Graubard (1932) and Redfield (1932) for chromosome II, and Redfield (1930) for chromosome III. The analyses are given in Table I.

TABLE I

Author	Chromosome	Region	Percentage of configurations with chiasmata					Map distance to 1st chiasma	Percentage to 2nd chiasma
			0	1	2	3	4		
Morgan (1933)	X (2623 flies)	b ₁ -f	78.96	21.04	—	—	—	10.52	0
		b ₁ -g	54.71	44.07	1.22	—	—	23.26	1.22
		b ₁ -v	36.79	57.72	5.49	—	—	34.35	5.49
		b ₁ -c ₁	18.57	62.52	17.99	0.92	—	50.63	18.91
		b ₁ -c ₂	12.39	58.64	27.75	1.22	—	58.90	28.97
		b ₁ -e ₁	7.51	48.88	41.17	1.83	0.61	69.58	43.61
Anderson & Rhoades (1931)	X (26,908 flies)	f-g	77.46	22.54	—	—	—	11.27	0
		f-v	55.91	43.14	0.95	—	—	22.52	0.95
		f-c ₁	32.79	60.04	7.14	0.03	—	37.21	7.17
		f-c ₂	21.94	65.20	12.62	0.24	—	45.58	12.86
		f-e ₁	12.83	64.41	22.10	0.65	—	55.29	22.75
		f-s ₁	7.18	62.88	28.72	1.10	0.12	62.05	29.94
Graubard (1932) 25° C.	II left (5284 flies)	s.f.-a ₁	17.97	76.97	4.92	0.33	—	43.81	5.25
		s.f.-t ₁	35.72	62.81	1.44	—	—	32.85	1.44
		s.f.-b	88.28	11.69	—	—	—	5.85	0
	II right (5284 flies)	s.f.-c ₁	95.55	4.43	—	—	—	2.22	0
		s.f.-v ₁	73.52	26.00	0.45	—	—	13.45	0.45
		s.f.-h ₁	65.01	33.00	1.66	—	—	18.31	1.66
Redfield (1932)	II left (9190 flies)	s.f.-s ₁	13.85	75.07	10.75	0.33	—	48.79	11.08
		s.f.-a ₁	19.63	75.01	5.35	—	—	42.86	5.35
		s.f.-d ₁	36.34	61.52	2.13	—	—	32.85	2.13
	II right (9603 flies)	s.f.-b	88.58	11.39	—	—	—	5.70	0
		s.f.-c ₁	61.60	38.37	—	—	—	19.19	0
		s.f.-p ₁	24.83	67.73	7.41	—	—	41.28	7.41
Redfield (1930)	III left (4919 flies)	s.f.-s ₁	16.69	72.95	10.08	0.25	—	46.94	10.33
		s.f.-r ₁	19.25	73.96	6.38	0.41	—	43.97	6.80
		s.f.-h	62.51	36.02	1.46	—	—	19.48	1.46
	III right (4919 flies)	s.f.-t ₁	94.43	5.57	—	—	—	2.79	0
		s.f.-s ₁	95.00	5.00	—	—	—	2.50	0
		s.f.-c ₁	95.00	5.00	—	—	—	2.50	0
		s.f.-s ₂	70.77	28.71	0.53	—	—	14.88	0.53
		s.f.-e ₁	53.57	44.52	1.91	—	—	24.17	1.91
		s.f.-c ₂	4.17	75.29	19.92	0.65	—	58.53	21.57

In chromosomes II and III s.f. = spindle attachment. The spindle attachment is marked by the gene *p₁* (for genetical purposes) in chromosome II, and in chromosome III is taken as occurring half-way between *s₁* and *c₁*.

Anderson & Rhoades's data concern the X-chromosome marked from *f* to *s₁* and involve 26,908 flies, whereas Morgan's data, while only involving 2623 flies, has the chromosome marked from *b₁* to *e₁*. The two sets of data give very similar results, with the latter showing rather more double crossing-over than the former. Graubard's (at 25°C.) and Redfield's data (control series for the two separate arms) give closely parallel

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results for chromosome II. They suffer from the defect of involving some long unmarked regions in which undetected double crossing-over will have occurred. Redfield's data for chromosome III (control series for triploids with one of the three chromosomes marked) are also deficient in the same way.

Table I shows the frequency of formation of 0, 1, 2, etc., chiasmata in the region between the spindle fibre and each gene in turn. Some assumption had to be made in each case as to the position of the spindle attachment. In the case of the X , b_1 is genetically practically coincident with the spindle attachment, and so the analysis of Morgan's data is valid. In the case of the other X data b_1 was not followed, and so the leftmost point was f . This has been assumed to represent the spindle fibre. The fact that, on analysis, they show good agreement with Morgan's data shows that this assumption does not vitiate the analysis, and involving, as they do, more than 10 times as many flies as Morgan's figures they are of great value.

In chromosome II the spindle fibre is assumed to lie at the locus of p_r and in chromosome III half-way between s_1 and c_n . In the latter case half the cross-overs in this region were counted as occurring on the left and half on the right of the spindle attachment. Neither of these assumptions is strictly correct and both do, in fact, result in certain complications which will be considered below.

In Fig. 2 are plotted the curves showing the relations between the map distance of various loci from the spindle attachment and the frequency of formation of two chiasmata in these regions. The most striking feature is that the curve for the X -chromosome is higher than the others. The curve for the left arm of chromosome II also appears to be lower than that which fits the points from the right arm of this chromosome and both arms of chromosome III. It is also quite striking that in the regions near the spindle attachment, the II right and III joint curve is higher than either of the others.

The latter feature is attributable to the assumptions made about the locus of the spindle attachment in the cases of chromosomes II and III. In chromosome II, p_r is rather to the left of the spindle fibre. Hence, in the present analysis, the region p_r -s.f., really in the left arm, is included in the right arm. As crossing-over in the two arms is independent the comparatively large double crossing-over involving the region p_r -s.f. and regions close to the spindle attachment, is included in the right arm. Including the former region in the right arm will hence spuriously increase the amount of double crossing-over apparently occurring in that

arm. This is clearly responsible for the excess double crossing-over near the spindle fibre in Fig. 2. On the other hand, the left arm of chromo-

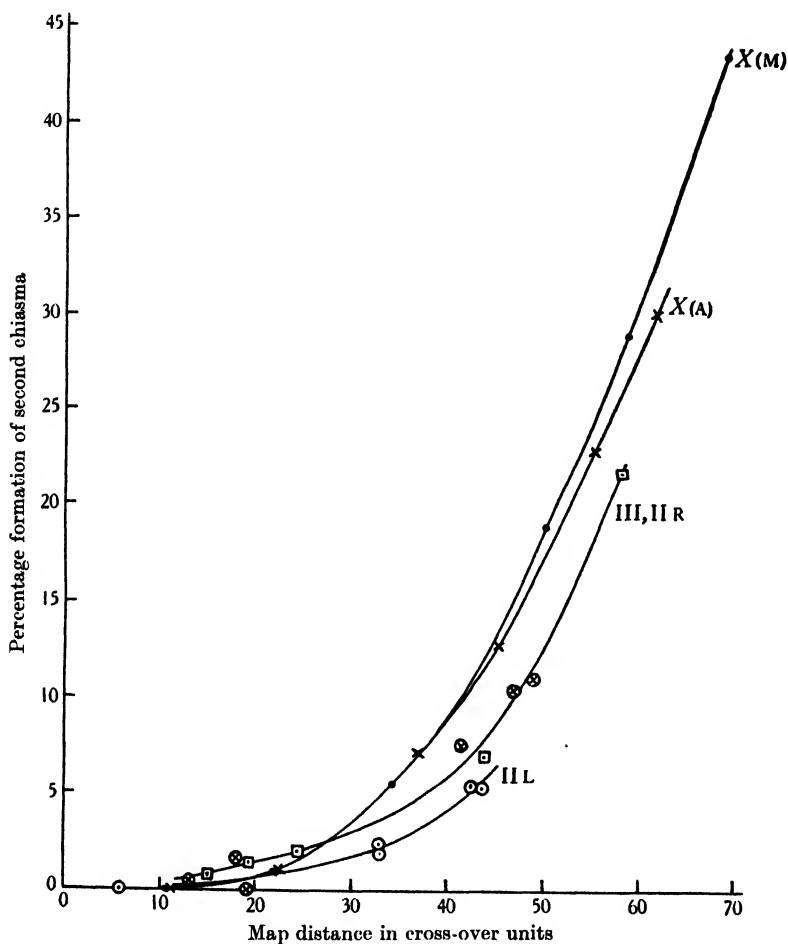


Fig. 2. Curves showing the frequencies of formation of two chiasmata for regions of different genetical map lengths in the three long chromosomes of *Drosophila*. The curves all have the spindle attachment as origin.

X (M), X-chromosome from Morgan's (1933) data.

X (A), X-chromosome from Anderson & Rhoades' (1931) data.

III, II L, joint curve for the third chromosome and the right arm of the second chromosome. Data from Redfield (1930, 1932) and Graubard (1932).

II R, right arm of chromosome II. Data from Redfield (1932) and Graubard (1932).

some II will not show this excess double crossing-over and so should be lower in general than the right-arm curve. More particularly it should not be as high near the spindle attachment. This is borne out by the

observations. Hence the left-arm curve is a more accurate representation of what really happens than is the right-arm curve. It would appear that practically the whole of the difference between the left- and right-arm curves could be attributed to this somewhat inaccurate assumption of the spindle fibre lying at p_r .

In chromosome III the spindle fibre was assumed to lie in the middle of Redfield's region 4, and all cross-overs, including doubles, which involved this region were assumed to be half ascribable to the region on each side of the spindle attachment. Now, in the case of the double cross-overs, it is clear that, as a result of the independence of the arms, where region 4 and a left-arm segment were involved, the part of region 4 concerned would, in the majority of cases, be that to the right of the spindle fibre and vice versa. Hence the method of analysis used would tend to show excess double crossing-over, and this would be particularly noticeable in the spindle attachment regions, just as in the right arm of chromosome II. This accounts for the observed excess just as in the case of chromosome II.

The other difficulty in the way of correct interpretation is due to the long unmarked regions of chromosomes II and III, which allow of undetected double crossing-over. By the use of the correction curves given by Bridges & Morgan (1923) for chromosome III it appears that, with the genes used, about 1.25 units of crossing-over have been missed from this cause in each arm. As an undetected double cross-over involves a loss from the record of half the crossing-over to which that case of formation of two chiasmata would give rise, about 0.625 per cent. of the strands were double cross-over not recorded as such. Thus the end-point of the curve for chromosome III, in Fig. 2, should be moved upwards by 2.5 units on the ordinate (each unit is 1 per cent. formation of a second chiasma). This is not sufficient to account for the difference between the *X*-chromosome and chromosome III curves. Furthermore, it must be remembered that the latter curve has been artificially raised to some extent by the assumptions about the spindle fibre.

The curves for chromosome II are probably too low by the same amount as chromosome III from the cause of undetected doubles. Again, however, the right-arm curve is too high as a result of the incorrect spindle attachment assumptions, and so, like chromosome III, it cannot be judged to be showing the same relation between map distance and double crossing-over as the *X*-chromosome. The left-arm curve of chromosome II is not artificially raised by the spindle fibre assumption and is too low by about 2.5 per cent. at the end as a result of undetected double

cross-overs. This is not nearly sufficient to account for the difference between it and the X-chromosome curve.

Thus of the two differences noted in the curves of Fig. 2, one, the difference between the curve of chromosome II left and chromosome III, which includes II right, is false and may be ascribed to the incorrect

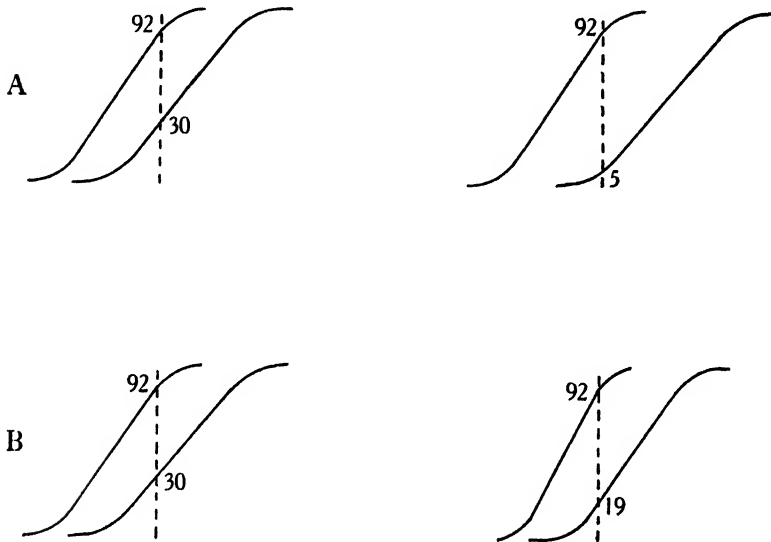


Fig. 3. Diagram showing the effect of varying relations between (A) cytological length and interference and (B) different "concentrations" of the frequency curve of formation of the first chiasma on the frequency of formation of two chiasmata in regions of a given map distance. The curves are those relating the genetical and cytological distances of various loci from the chosen origin and so are integrals of the frequency distribution of crossing-over along the chromosome. The leftmost curve in each diagram is that of the first chiasma and the right most that of the second chiasma. The figures are the frequencies of occurrence of a chiasma (i.e. twice the cross-over value) given by each curve at its intersection with the dotted line. This is constant, at 92, for the first chiasma and varies from the second chiasma curve. It shows that either increased cytological length of interference as in A or increased concentration, i.e. localization, of the first chiasma as in B, leads to a decrease of the frequency of formation of two chiasmata over any given map distance.

assumptions made in the analysis. The other difference, that between the X-chromosome and the autosomes, is real.

This receives some confirmation from the curves obtained if the genes nearest the ends of the chromosomes are taken as the origins instead of the spindle attachments. The curves of chromosomes II and III are then coincident, but that for the X-chromosome is higher.

The difference between the X-chromosome and the autosomes is further evidence for the original argument that the regional frequency

of crossing-over is not constant along the chromosome. It also shows that the determining mechanism varies between the chromosomes.

The observed difference may be expressed in a somewhat different way. Consider points at a constant map distance from the spindle attachment but in different chromosomes. In the *X*-chromosome there will be more of this map distance accounted for by double crossing-over than in the other chromosomes. This may result from either or both of two things, viz. (a) that the first chiasma occurs over a more concentrated range in the chromosomes II and III than in the *X*, (b) the physical distance over which interference of any given strength occurs is shorter in the *X*-chromosome than in the others. Fig. 3 illustrates these points. The curves in each section of the figure are the integrals of the curves which would represent the regional distribution of the first and second chiasmata. In other words the curves represent the relation between genetical and cytological distances from some origin. The vertical lines cut the first chiasma curve at a fixed level. The ordinate of the second chiasma curve at its intersection with the vertical line illustrates the effect of changing concentration of the curves and changing distance of interference.

IV. THE REGIONAL DISTRIBUTION OF THE FIRST CHIASMA

Taking the first chiasma to be the one nearest the spindle attachment, it is possible to construct curves for the different chromosomes to show the relation between cytological distance and frequency of crossing-over due to the first chiasma. The genetical distance due to the first chiasma is obtained by summing the percentages of cases in which at least one chiasma is formed, and dividing by two, as 100 per cent. formation of one chiasma corresponds to 50 per cent. genetical crossing-over. The data of Table I allow of the construction of curves which give the relations between total map distance from the spindle attachment and that fraction of it ascribable to the formation of the first chiasma. With such curves we can convert any map distance to the corresponding frequency of crossing-over due to the formation of the first chiasma. All the first chiasma cross-over frequencies used below have been obtained from published map distances (Sansome & Philp, 1932) by the use of such curves.

The cytological data are obtained from the papers of Muller & Painter (1932) on the *X*-chromosome, Dobzhansky (1930) on chromosome II and Dobzhansky (1929) for chromosome III (see Fig. 1). These authors determined the cytological and genetical locations of various chromosome

breakages induced by irradiation. The genetical distances of the breaks from the spindle attachments may be converted into first chiasma cross-over frequencies by the correction curves described above. The cytological distances may be measured from the diagrams of the mitotic metaphase chromosomes given by the authors (Table II). These cytological positions as determined from the oögonial metaphase chromosomes are open to some error, and it has been found that the diagrams given by the authors are not always absolutely consistent (cf. Dobzhansky, 1930, 1931). However, these discrepancies, while invalidating detailed comparisons of the curves, do not affect the broad differences to be observed between the chromosomes. The necessary comparative lengths of the mitotic chromosomes were kindly supplied by Dr Dobzhansky.

TABLE II

Chr. X		Chr. II L		Chr. II R		Chr. III L		Chr. III R	
G.	C.	G.	C.	G.	C.	G.	C.	G.	C.
0	0	0	0	0	0	0	0	0	0
0	9.75	7.5	17.87	0.4	2.55	0.5	4.12	2.5	5.3
0	12.02	37.2	22.33	0.4	3.83	5.4	18.85	49.4	28.87
0.02	19.49	41.3	22.97	0.4	5.11	5.5	18.85		
0.02	20.79			15.6	15.95				
13.2	23.71			39.5	17.55				
38.8	28.91			39.9	18.19				
49.3	34.43								

G.=first chiasma map distance from spindle attachment. C.=oögonial mitotic cytological distance from spindle attachment in arbitrary units.

The curves obtained by plotting first chiasma cross-over frequencies against mitotic cytological distance for the three long chromosomes are given in Fig. 4. These curves must tend to a genetical upper limit of 50 units. As the first chiasma cross-over frequency is clearly the sum of the crossing-over in all the sections of chromosome between the point under consideration and the spindle attachment, the curves given in Fig. 4 *a* are the integrals of the curves which would show the regional distribution of first chiasma formation. These distribution curves could be obtained by differentiation of the integrals or geometrically by determining the slope of the tangent to the curve at various levels. If the slope of the tangent is plotted against cytological distance, the distribution curves are obtained. This has been attempted in Fig. 4 *b*, but the accuracy of the integral curves is not sufficiently great to justify the placing of much faith in the derived curves.

Similar curves based on crossing-over due to the second chiasma could be constructed, but only the X-chromosome is long enough to give

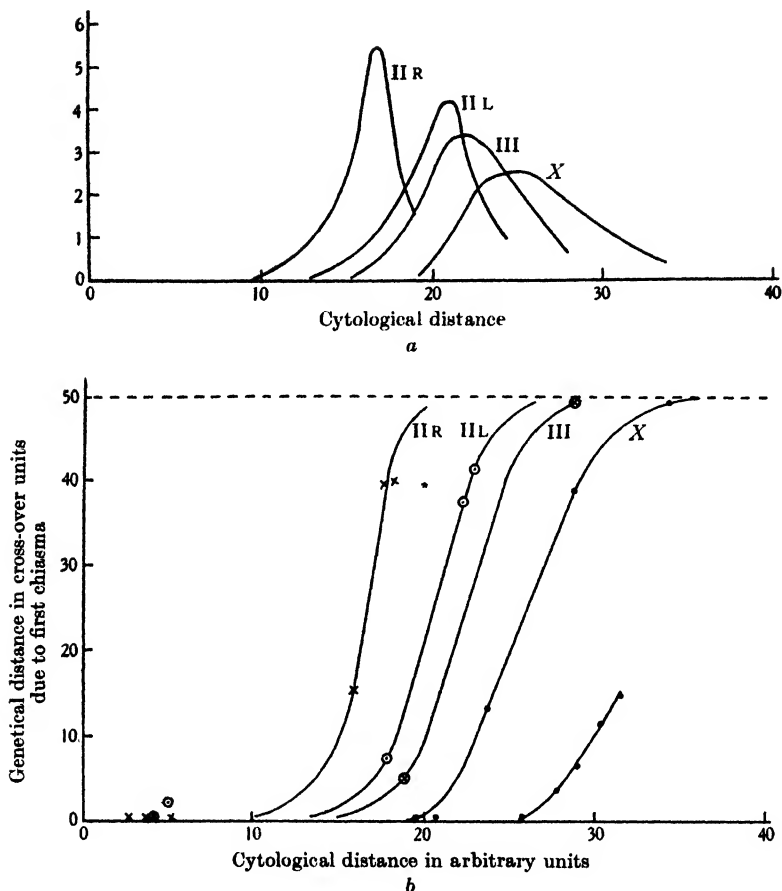


Fig. 4. (a) Curves relating the frequency of crossing-over produced by the first chiasma to mitotic metaphase cytological distance, for regions originating at the spindle attachment, in the long chromosomes of *Drosophila*. These are the integrals of the curves of frequency distribution of the first chiasma along the cytological chromosome. The short curve on the right is that for the second chiasma in the X-chromosome. Note that its slope is less than that of the first chiasma curve in the X. The upper limit of 50 units to which the curves tend is the amount of crossing-over to which the constant formation of one chiasma gives rise. (b) The curves of distributional frequency of formation of the first chiasma along the cytological chromosome. They are derived from the curves of Fig. 4 a by geometrical differentiation by measuring the slopes of the tangents to the above curves at various points. Their accuracy is open to question.

In each figure the second chromosome has two curves, II L for the left and II R for the right arm.

a useful curve. This is plotted in Fig. 4 *a*, and it has a slope less than that of the *X*-chromosome first chiasma curve.

It is clear from Fig. 4 *b* that the curve for the *X*-chromosome has its maximum, or, in the integral curve, point of maximum slope, at a distance farther from the spindle attachment than does either chromosome II or III. The maximum appears to be lower in the *X*-chromosome than in any other, but the significance of this is doubtful. The difference between the left- and right-arm curves of chromosome II is probably due to the spindle attachment having been assumed to be median, whereas actually this is not quite true (Kaufman, 1934). The spindle attachment of chromosome III may not be median either, but the number of points on this chromosome is not sufficient to show up any difference between the two arms. Both the arms of chromosome II give curves whose maxima are nearer the spindle fibre than that of chromosome III. This is apparently connected with the shorter length of this chromosome. The distance from the spindle attachment of the maximum of the derived curve appears to be dependent on the length of the chromosome inasmuch as the *X*-chromosome has it farthest away, then chromosome III and, closest of all, chromosome II. As the *X* has a terminal spindle attachment it ranks as one chromosome arm, but the other two chromosomes both have median attachments, their two arms rank separately for this analysis and so the sizes of chromosome arms fall in the order, given also by the maxima, of *X*, III, II.

One of the points to which our interest is particularly directed is that of the relative "concentration" of crossing-over in the different chromosome arms. This is of importance in view of the varying relations of double crossing-over and map distance in the different chromosomes as shown in the previous section. The greater the concentration of crossing-over or the lower the variance or spread of the curve in any region, the higher will be the maximum, provided that the general shape of the curve does not radically change. If we consider the integrals of the distribution curves, such as were obtained in Fig. 4 *a*, the height of the maximum of the distribution curves may be measured by the slope of the tangent at the point of maximum slope. Thus it may be stated that the greater the slope of the centre region of the integral curve, the greater the concentration of crossing-over in that chromosome. The data from cytological measurements on mitotic chromosomes are not accurate or numerous enough to allow of measurement of slope of the curve, and so of concentration of crossing-over. There is, however, another type of cytological observation which may be utilized for this purpose.

222 *The Determination of Position in Crossing-over*

Painter (1934, 1935) and Mackensen (1935) have published drawings of the chromosomes of *Drosophila melanogaster* as seen in the salivary gland nuclei. These drawings also show the locations of certain genes in each of the chromosomes (Table III). Hence the salivary cytological

TABLE III

Chr. X		Chr. II L		Chr. II R		Chr. III L		Chr. III R	
G.	C.	G.	C.	G.	C.	G.	C.	G.	C.
0	0	0	0	0	0	0	0	0	0
.....	0.5	4.94	0	4.23
11.0	3.38	20.0	10.96	4.0	6.62	2.0	10.22
13.3	5.20	6.5	6.43	41.1	16.68	5.8	7.60
15.7	5.52	39.8	16.86	45.1	19.31	21.1	13.83	13.8	16.21
20.0	7.93	48.5	20.09	42.5	20.74	21.2	18.18
23.5	8.45	27.9	20.86
32.6	{ 12.22	39.4	23.44
	{ 12.48	45.0	24.68
41.3	15.73
46.4	18.85
47.1	19.50
49.4	20.08
.....
50.0	20.28
50.0	21.38
50.0	21.71
50.0	23.01

G.=first chiasma map distance from spindle attachment. C.=salivary gland cytological distance from spindle attachment in arbitrary units. The dotted lines enclose the values used in the calculations of linear regression coefficients.

distance from the spindle attachment may be plotted against the first chiasma cross-over frequency in just the same way as the mitotic chromosome distance was used in Fig. 4 *a*. The relative lengths of the various chromosome arms have been taken from the figures of Painter (1934). Bridges (1935) has published figures differing slightly from Painter's in the lengths of these elements, but the calculations, described below, have been performed using his figures too. The results differ slightly from those based on Painter's data, but the significance of the differences of the behaviour of the different chromosomes remains the same.

Fig. 5 shows the resulting graphs. As before the curves must tend to an upper limit of 50 units, as that is the frequency of crossing-over that the regular formation of one chiasma will give. There is one striking difference between this figure and Fig. 4 *a*, viz. in the order of the curves. The curve for the sex-chromosome is now nearest the ordinate, followed by chromosome II and farthest away is chromosome III in which the arms give different curves. This is due to the fact that the inert regions of the chromosomes are not represented in the salivary gland chromosomes but do occur in those of the oogonial metaphase plates.

Thus the curves of Fig. 5 are moved to the left by an amount corresponding to the length of the inert region of that particular chromosome arm. It may be argued that since the inert regions are not genetically active they should be omitted, i.e. that the curves of Fig. 5 are more correct in position than those of Fig. 4 *a*. This cannot be upheld for four reasons, viz.:

(a) Crossing-over is known to occur in the inert regions of the X-chromosome in the male (Darlington, 1934; Philip, 1935).

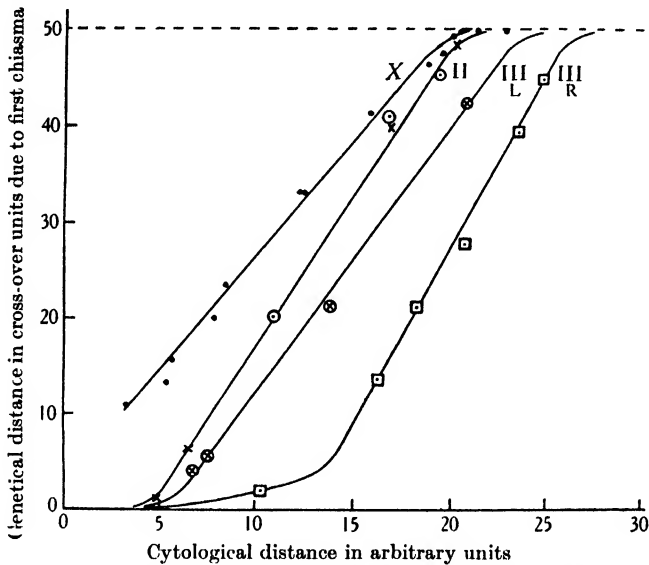


Fig. 5. These curves correspond to those of Fig. 4 *a*, but the cytological maps used in this case were those from salivary gland chromosomes. The third chromosome now shows two curves, one for each arm separately.

(b) The inert regions are present in the oogonial metaphase chromosomes and so are presumably present at the prophase of meiosis. It would then seem unreasonable to suppose that they had no effect on the distribution of crossing-over.

(c) Fig. 4 presents the curves in order of size of the chromosome arms. There is no apparent reason for the order of the curves in Fig. 5.

(d) If the X-chromosome line is continued downwards with the same slope, it cuts the ordinate before it cuts the abscissa. This means that, unless the X-chromosome line differs in shape from those of the other chromosomes, in curving downwards at the proximal end, crossing-over must occur in the inert region of the X-chromosome in the female as well as in this region in the male. Fig. 4, in which the inert regions are

represented, shows that the *X*-chromosome has frequency distribution curves of the same type as the other chromosomes and so the latter explanation must be adopted.

Unless it is assumed that there are interstitial inert regions in addition to those near the spindle attachments, the salivary gland curves of Fig. 5 do give a true representation of the slopes of the mid-regions and so of the concentration of crossing-over. If such interstitial inert regions are present they must be small and very regularly spaced along the sex-chromosome as the curve of this chromosome in Fig. 5 is very regular. This would appear to be an unlikely contingency, and so the curves of Fig. 5 may be taken as truly representing the integrals of the distribution curves but moved to the left by amounts dependent on the lengths of the distal inert regions.

The number of points on each of these curves allows of an accurate test of the similarity or otherwise of their maximum slopes. This can be done by the calculation of the regression coefficients of the first chiasma cross-over frequencies on salivary gland cytological distance, for each line separately. The coefficients may then be compared. For this purpose the portions of the curves which approximate to straight lines are used, as it is the linear regressions which are to be calculated. This is of course an approximation as the lines are really curves. The points at the ends of the curves must be omitted if clearly deviating from the straight lines. Only one point really raises any doubt as to whether it lies on the best fitting straight line for that chromosome arm or not. This is the point (10.2, 2) on the chromosome III right curve. Calculations showed that it could not be considered as lying on the same straight line as the points of this curve and so it was omitted. Two of the points at the upper end of the *X*-chromosome curve were omitted for the same reason.

The details of the calculation will be found in Fisher (1934). The regression coefficients are the slopes of the lines as estimated by the method of least squares. They are obtained from the formula

$$b = \frac{S(x - \bar{x})(y - \bar{y})}{S(x - \bar{x})^2},$$

where b is the linear regression coefficient, x is the independent variate, i.e. the cytological distance, and y the dependent variate, i.e. the cross-over frequency. \bar{x} and \bar{y} are the means of x and y . The variance of each regression coefficient is obtained from the formula

$$V_{(b)} = \frac{S(y - Y)^2}{(n - 2) S(x - \bar{x})^2},$$

where $V_{(b)}$ is the variance, x and y are as before, Y the calculated value of y and n the number of points on the line.

The coefficients and variances obtained are

Chromosome X	b	$V_{(b)}$	Degrees of freedom
II left	2.343508	0.003681	9
II right	3.103657	0.009075	1
III left	3.116348	0.266812	1
III right	2.725314	0.017070	2
	3.606680	0.048332	3

The number of degrees of freedom is given in each case by the number of points on the line, less two, as two parameters, the mean and regression coefficient, have been estimated.

The slope of any two lines may be compared by the calculation of the quantity t which is the ratio of the difference between the two regression coefficients and the square root of the sum of their variances. For this purpose the variance is calculated from the pooled sum of $(y - Y)^2$. This is expressed in the formula

$$t = \frac{b_1 - b_2}{\sqrt{s_1^2 + s_2^2 \left(\frac{1}{n_1 + n_2} \left(\frac{1}{S(x_1 - \bar{x}_1)^2} + \frac{1}{S(x_2 - \bar{x}_2)^2} \right) \right)}}$$

where $s^2 = S(y - Y)^2$, b_1 , s_1 , etc., belong to the first line and b_2 , s_2 , etc., to the second. The quantity t is then entered in the table of t (Fisher, 1934) for a number of degrees of freedom given by the sum of those appertaining to each individual coefficient. The probabilities of obtaining from a homogeneous population differences of the coefficients as large or larger than those found is obtainable from the table. The differences, variances of the differences, t statistics, degrees of freedom and probabilities are:

	$b_1 - b_2$	$V(b_1 - b_2)$	t	Degrees of freedom	P
X-II L	0.760149	0.016611	5.8977	10	Less than 0.01
X-II R	0.772840	0.060647	2.9955	10	0.02-0.01
X-III L	0.381806	0.015979	3.0204	11	0.02-0.01
X-III R	1.263172	0.037139	6.5544	12	Less than 0.01
II L-II R	0.012691	0.198337	0.0285	2	Greater than 0.90
II L-III L	0.378343	0.030881	2.1530	3	0.20-0.10
II L-III R	0.503023	0.060894	2.0384	4	0.20-0.10
II R-III L	0.391034	0.165591	0.9609	3	0.50-0.40
II R-III R	0.490332	0.201301	1.0929	4	0.40-0.30
III L-III R	0.818137	0.064643	3.4665	5	0.02

It is clear from these results that the X-chromosome curve has a slope lower than any of the others, while there is no evidence of differences among the rest except between III L and III R. Thus there is confirmation of the differences expected between the chromosomes. The X-chro-

mosome has a more scattered distribution of the first chiasma along its cytological length than do the rest, which appear to be very much alike. This may be expressed somewhat differently by saying that the frequency distribution curve of the first chiasma along the *X*-chromosome has a lower maximum, and hence higher variance than any of the other chromosome arms. The lower maximum clearly involves a higher variance as the total area of the curve is fixed, being in fact the amount of crossing-over given by the consistent formation of one chiasma. The meaning of the apparent difference between the two arms of chromosome III is worthy of further investigation.

The relation of the distribution of the first chiasma to the spindle attachment may now be stated more precisely. The frequency of formation of the first chiasma in any segment of the chromosome arm is very low near the spindle attachment, rising to a maximum at some distance away and then falling. The maximum occurs farther away from the spindle attachment as the length of the chromosome arm increases. The precise relation between the length of the chromosome arm and the situation of the maximum cannot be stated from the present data. There is, however, good evidence for the above generalization.

The *X*-chromosome distribution has a lower maximum and greater variance, or "spread", than those of the other chromosome arms. It seems likely that the general rule that the farther from the spindle attachment that the maximum lies, i.e. the longer the chromosome arm, the lower the maximum will be and hence the greater the variance. This would demand that chromosome III has lower maxima than chromosome II, but there is no convincing evidence for this at present.

These differences clearly account for at least part of the discrepancy between the double crossing-over and map distance relation curves of the *X* and other chromosomes as shown in Fig. 2. The next question to be considered is whether interference differences are involved.

V. INTERFERENCE. THE RELATION BETWEEN THE FIRST AND SECOND CHIASMATA

This question is not capable of such exact treatment as that of the distribution of the first chiasma, on account of insufficient data. Perhaps the most profitable line of approach is to consider the relation between the degree of interference over any region and the cytological length of that segment.

The first difficulty which is encountered is that of finding a suitable measure of interference. The coincidence value was one of the earliest

statistics relating to crossing-over to be calculated. Its reciprocal can be defined as a consistent measure of interference which is unity in the absence of interference and increases to infinity as interference increases. There is one great drawback to this measure for our purpose, viz. it must always be based on two regions of the chromosome, each often 10 or more units long. The resulting value must then be related to the distance between the mid-points of the regions, with consequent inaccuracies.

There is a measure of interference which is a function of the distance between two loci. This is more desirable for the present purpose as it can be related to the cytological distance between the two loci to give a curve, whose only inaccuracies are those inherent in the data and not introduced by the method of calculation. This measure of interference was proposed by Anderson & Rhoades (1931) and is calculated from the formula

$$I = \frac{y - 0.5(1 - e^{-2x})}{x - 0.5(1 - e^{-2x})},$$

where I is the interference value, x the map distance between the two loci, and y the recombination value of the two loci. The formula is derived from a consideration of the relations of the observed curve of recombination and map distance to those that would be obtained in the absence of interference (formula $y = x - 0.5(1 - e^{-2x})$ from Haldane, 1918) and with complete interference all along the chromosome ($y = x$). I is 1 for complete interference and falls as interference falls. The exact interpretation of the value of I is impossible, but, unless the relation between the distributions of the first and second chiasmata are completely different in different chromosomes or parts of chromosomes, and what evidence there is is strongly against this assumption, similar values of I for a given physical distance would appear to indicate similar situations. Coincident curves for the relations between I and cytological distance must indicate similarity.

Anderson & Rhoades calculated the lines which show how the value of I is related to map distance between the two loci concerned in the X-chromosome. The derived lines were straight and varied in position with the point that had been taken as the origin. This led these authors to the conclusion that interference varies along the X-chromosome. If, however, their values of I are plotted against the cytological distance, based on salivary gland chromosomes, between the loci all the lines coincide with the exception of that originating at the locus of s_6 . This

discrepancy may be ascribed to the situation of s_c in a part of the chromosome where crossing-over is comparatively rare (see Fig. 1). All the other genes used in the experiment, $f-e_c$, occur in the mid-region of the chromosome where crossing-over is fairly evenly distributed. It is clear that the inclusion of a region with no crossing-over would push the interference-cytological distance line to the right, and a region of sparse crossing-over must have the same effect but to a reduced degree. There is a possibility, too, that the occurrence of triple crossing-over affects the values of I in this region, as nearly 75 per cent. of the triples involve the region s_c-e_c .

In view of these considerations it seems quite legitimate to conclude that the relation between interference and physical distance is constant along the *X*-chromosome.

The data available for the other chromosomes are not so good as those for the *X*. Some data are obtainable from Bridges & Morgan (1923) for chromosome III. They have given curves relating the difference between recombination and map distance to map distance, starting at a number of different loci in both arms of the chromosome. From such curves values of I may be computed. Regions including the spindle attachment could not be used, as crossing-over in different arms is independent. Also regions including portions with sparse crossing-over give erroneous results for reasons reviewed above. This is further demonstrated by the anomalous results obtained when the gene p , close to the spindle attachment in the right arm, is used as one of the loci. Values of I for portions of the chromosome originating at the genes r_a , d_v , h , s_a , D and H were used. The points obtained when the values obtained were plotted against salivary cytological distances are shown in Fig. 6. Of the eleven points, eight lie on the same line as the *X*-chromosome values, two deviate to the right and one to the left. The two right deviations are given by the only two points originating at r_a and the left deviate by the point using h as the origin. A scrutiny of Bridges & Morgan's original data might perhaps reveal some reason for these deviations. Long unmarked regions near to r_a would account for its points deviating to the right. However, the other eight points give so good a fit to the *X*-chromosome line that it allows one to conclude that the interference relations of both the *X*-chromosome and chromosome III are the same. No good data exist for chromosome II. Those of Graubard and Redfield are unusable for the two reasons that both involve genes in regions of sparse crossing-over and also long unmarked regions in which undetectable double crossing-over will occur.

There is some confirmation to be obtained from the results of Schweitzer (1935), who estimated the length of chromosome over which interference just vanishes, using various loci as the origin. The measure of physical length used was what he terms the "leptotene unit" based on "leptotene maps" of the chromosomes. These maps were obtained from regional mutation frequencies and agree well enough with the salivary gland maps, including the absence of the inert regions, to be

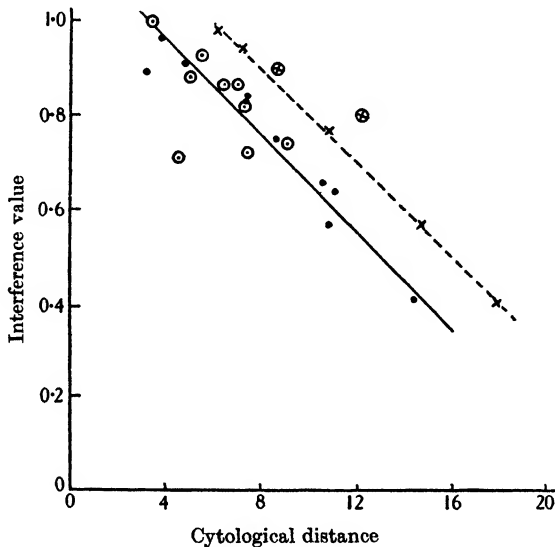


Fig. 6. The relation between the value of I , the interference measure (see in text) and salivary gland chromosome distance between pairs of loci in chromosomes X and III . The dots are from pairs of loci in the X -chromosome excepting those involving s_e and all fall on one line. The crosses are the points from pairs, one of which is s_e and fall on a somewhat different line (dotted). The circles with dots are the chromosome III determinations and agree well with the main body of points from the X -chromosome. The circles with crosses are two chromosome III points both deviating to the right of the line and both involving the locus of r_a .

compared with them. This author found that with the exception of the region s_e (or y)- e_e , all regions of the X -chromosome show absence of interference at a minimum distance of 50–55 leptotene units. This is detailed confirmation of the results obtained using Anderson & Rhoades' interference formula. On the other hand, Schweitzer found that the minimum distance of no interference in chromosome II is not constant. His Fig. 4 shows it to have a value of 50 leptotene units at the ends of the chromosome and falling to about 25 in the spindle attachment region. It will be remembered that this author came to the conclusion that the

spindle attachment had no effect on interference.¹ It is then to be presumed that he was impartial as to whether the regions he was considering lay on the same or different sides of this point. Now it has been pointed out in an earlier section that crossing-over must be considered to be independent in the two arms and so Schweitzer's results become explicable. The low values for the central regions are due to this faulty assumption of the lack of effect of the spindle attachment. The only regions giving the true minimum distance of no interference will be the end-regions. These give values of apparently 50 leptotene units, as did the X-chromosome. Thus Schweitzer's results confirm rather than disprove the validity of the conclusion that the interference-physical distance relations are the same in all chromosomes and parts of chromosomes.

With interference constant in all the chromosomes, no part of the difference between the curves of the X and other chromosomes in Fig. 2 can be ascribed to this cause. They must be completely accounted for by the differences in spread, or variance, of the chiasma frequency distributions as described in the previous section.

VI. GENERAL DISCUSSION OF THE HYPOTHESIS

All the data examined are in agreement with and easily interpretable by the hypothesis that crossing-over in any region is dependent on the distance of that region from the spindle attachment. The precise relation between regional cross-over frequency and distance from the spindle attachment is dependent on two possible variables, viz. the relation of the distribution of the first chiasma to the spindle attachment and the relation of the second chiasma to the first. The latter appears to be actually constant both within and between chromosomes (section V). The former is variable in a way which may now be specified with some accuracy (see section IV).

Some further consequences of the hypothesis may now be examined. The region near the spindle attachment is a region of very low crossing-over. Hence any absolute increase of crossing-over will be relatively greater in this region than elsewhere. In particular, if the whole distribution curve of the first chiasma is moved towards the spindle attachment there will be marked increases in crossing-over in the spindle-attachment regions and little, if any, distally along the chromosomes. This is what is found to be the case as a result of high or low tem-

¹ Schweitzer also reaches the conclusion that crossing-over is independent in the two arms. This is difficult to reconcile with his earlier conclusions in the same paper and with his method of working.

peratures and X-irradiation (Plough, 1917; Graubard, 1932; Muller, 1925). In general, on the present hypothesis, the spindle attachment regions should show more variation in crossing-over than more distal parts of the chromosomes. Gowen (1919) reached the conclusion that this was the case in chromosome III from a general statistical consideration of experimental crossing-over results.

There is another feature worthy of note. The inert regions are, on the present hypothesis, regions which would, being close to the spindle attachment, have very low crossing-over. This would appear to indicate a causal relation between low crossing-over and inertness (cf. Muller, 1918). Since the right arm of chromosome III shows an active region with low crossing-over near the spindle attachment, it must be concluded that, on this view, inertness results from low crossing-over, and not the reverse.

The present results also bear on the question of how the short chromosome IV pairs. From section IV it appears that the shorter the chromosome arm concerned the more "concentrated" is the distribution of the first chiasma formation frequency along the physical map. Hence in the short chromosome IV the first chiasma should always form in a very localized position, presumably near the distal end. Furthermore, as this chromosome is shorter than the physical distance of complete interference, as determined from the three long chromosomes, two chiasmata should never occur. This removes the difficulty in linking up the *Drosophila* breeding results, which show practically no crossing-over in this chromosome, with the observations of Darlington & Dark (1932) who found that, in grasshoppers, even the extremely short chromosomes always formed a chiasma. Genes proximal to the chiasma would seldom show crossing-over among themselves, and with a chiasma localized at the distal end the chance of finding a gene distal to it is small. Hence neither visible crossing-over nor equational non-disjunction would be expected except as an extreme rarity.

Finally, the results of the present analysis confirm the postulated formation series of the chiasmata along the chromosomes, starting at the spindle attachment. The observed orderly distribution of the chiasmata with regard to the spindle attachment could hardly be obtained without such a seriation. Furthermore, it will be seen from Fig. 4 *a* that the curve for the second chiasma has a lower slope than that for the first chiasma in the X-chromosome. If the second chiasma formed first or the order of formation was not definite, this could not be so. The lower slope of the second chiasma curve means that the corresponding fre-

quency distribution curve has a greater variance or spread than that of the first chiasma. This is to be attributed to the second chiasma being subject to two kinds of variation, that in the position of the first chiasma with regard to the spindle and that of the second chiasma with regard to the position of the first. The first chiasma is not affected by this second type of variation. If the so-called second chiasma formed first the situation would be reversed and the second chiasma curve would have a steeper slope than that of the first. If the order of formation was indeterminate the slopes would of necessity be equal. This spacial order of formation of the chiasmata demands a corresponding temporal order of formation. Hence chiasma formation must start at the spindle attachment in *Drosophila* as it does in Darlington's *Fritillaria*.

VII. SUMMARY

1. Both genetical and cytological evidence indicate that crossing-over is not constant per cytological unit distance along chromosomes, particularly in those of *Drosophila melanogaster*. This must mean that some localized determining mechanism exists in the chromosomes.

2. Comparison of the genetical and cytological chromosome maps of *Drosophila* and genetical data on the effect of the spindle attachment on cross-over values indicates that the spindle attachment is the site of this mechanism. The two arms of the V-shaped chromosomes might then be considered to be independent in their crossing-over. This is shown to be the case from genetical data.

3. The frequency of crossing-over and chiasma formation in any region may then be expressed as a function of the distance of that region from the spindle attachment. Any analysis must be in terms of chiasmata, i.e. points of crossing-over, as it is their relations with the spindle attachment that may vary. The observed crossing-over data must be transformed into chiasma frequency data for the purpose of such analysis. Considering the proximal two chiasmata, their position depends on two potential variables: (a) the frequency distribution of the formation of the first chiasma with respect to the spindle attachment, and (b) the relation between the positions of the first and second chiasmata, i.e. interference. The third and subsequent chiasmata are not considered on account of scanty data.

4. The distributions of the chiasmata cannot be the same in all the chromosomes, as the frequency of double crossing-over for any given map distance from the spindle attachment is not constant for different chromosomes.

5. The frequency of crossing-over resulting from the formation of the first or proximal chiasma per cytological unit of distance falls on a curve which is low near the spindle attachment and in the distal regions and with a maximum at some intermediate point. The distance of the maximum from the spindle attachment appears to be positively correlated with the length of the chromosome arm. The nearer the maximum to the spindle attachment the higher it is and the less is the variance, or spread, of the curve.

6. The data on the relations between the degree of interference between two points and their cytological distance apart are not complete. They do, however, definitely indicate that for points of equal cytological distance apart, in regions with nearly equal crossing-over, the interference value is constant both within and between chromosomes.

7. Thus the frequency of crossing-over in any region is dependent on both its distance from the spindle attachment and the length of the chromosome arm.

8. These results lead to the general conclusions that:

(a) Crossing-over frequencies will be most variable and liable to the greatest upset in the spindle attachment region, as has been shown to be the case.

(b) Inertness of portions of the chromosomes is conditioned by low crossing-over.

(c) The short fourth chromosome may always form a chiasma, but genes on it will seldom show crossing-over because of the extreme localization of the site of the chiasma.

9. These results support the view that in *Drosophila melanogaster* crossing-over and chiasma formation commence at the spindle attachment and proceed regularly along the chromosome arm. The first-formed chiasma is the proximal one.

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THE GENETIC STRUCTURE OF *TULIPA*

I. A CHROMOSOME SURVEY

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(With Eighteen Text-figures and One Diagram)

I. INTRODUCTION

IN 1927 the late W. C. F. Newton published a survey of *Tulipa* in which he showed the bearing of his chromosome studies on the classification of the genus. His conclusions have been amplified in a more general account by Sir Daniel Hall (1929, 1936). Since then a general genetic analysis has been undertaken by several workers in this Institution, using the methods of chromosome study and experimental breeding, the first by Dr C. D. Darlington and the writers, the second by Dr J. Philp.

The results of this work are now becoming available and will be published in a series of articles. In these we shall attempt to show how the methods of reproduction of a natural group determine its division into species and the kinds of species into which it is divided.

Provided that the experimenters can afford to wait five years for each generation, no more favourable material is to be found. The ornamental properties of the species have led to an accurate description of their variation, which is conspicuous to any observer. Many of the species cross readily and have apparently crossed in nature. The chromosomes are large and their structure can be accurately described at meiosis as well as mitosis. It is therefore possible to relate genetic variation with chromosome behaviour in greater detail than in other genera where similar studies have been attempted.

II. METHODS

The material upon which this article is based was obtained from the John Innes Institution's collection of tulips.

Root tips of all the species and varieties examined were fixed in 2 BE (La Cour, 1931). Divisions are most frequent in roots taken from the bulb a few weeks after planting. In order to shorten the usual process of dehydration, a modification of Randolph's method (1935) was used. After fixing for 24 hours and washing for half an hour, the material was

passed straight into 70 per cent. alcohol and left for 24 hours. It was then taken through absolute alcohol and chloroform into wax in the usual way.

Sections were cut at $24-30\mu$ and left for 24 hours to dry on the hot plate, for, unless thoroughly dried, sections of this thickness are liable to fall off the slide during the staining process. Newton's gentian-violet method was used as the stain, the saturated solution being slightly diluted and the slides left in for 3-4 min.

For meiosis and pollen grain divisions, smears were used wherever possible. The pollen mother cells smear very easily, but the pollen grains have developed a brittle wall at the time of division, and this is easily crushed by pressure of the knife. Furthermore, the anthers are usually very dry, so that the pollen grains will not spread out or stick to the slide. By cutting the anthers into small pieces and using very little pressure with the knife, smears of undamaged pollen grains were obtained.

In the triploids the number of dead pollen grains is so great that they prevent the living ones from sticking to the slide. This difficulty can be overcome if the anthers are crushed into a drop of albumen slide fixative, and then spread over the slide.

The best fixative for meiosis was medium Flemming, but for the pollen grains 2 BE (La Cour, 1931) gave better results.

For staining the pollen mother cells at all stages except pachytene a weak stain (0.1 per cent.) was used for about 5 min. The chromosomes at meiosis take up the stain very readily, and it was almost impossible to avoid over-staining if a higher concentration was used. For pachytene and pollen grain divisions, the root-tip stain gave better results.

We found it was possible to preserve a bud in water for a few days if it was too young at the time of opening the bulb. This was only feasible if the pollen mother cell had reached pachytene and had separated, for pachytene itself is a long stage which may last for several weeks. If an anther examined in acetocarmine contained pollen mother cells at pachytene and early diplotene, and later stages were required, the bud was wrapped in blotting paper and placed in the neck of a small bottle so that it remained moist but not too wet. If kept at $60-65^{\circ}$ F. overnight, the later stages of meiosis could be obtained the next morning. After 2 or 3 days, however, the anthers die and a rot sets in. Nevertheless, a great deal of wastage may be avoided in this way.

III. DATES OF DIVISION

Meiosis in the embryo-sac takes place just before the flower opens (Newton, 1927). In the anthers, however, it occurs several months before, in most species while the bud is still enclosed within the bulb. To obtain the bud the bulb must therefore be destroyed. In those species in which meiosis does not occur until February or March, the bud is to be found in the stem, usually just below the ground.

All members of a clone which have been stored under similar conditions undergo meiosis within a few days of one another. All the anthers in a flower are the same age, but within an anther there is a slight difference between one end and the other. Although pachytene may last for several weeks, the later stages of meiosis are completed in 2 or 3 days, so that the time during which preparations of these stages may be obtained is very limited. Bulbs that have been planted are as a rule a week or so in advance of those that have been kept dry. There is also a slight variation in date from year to year.

The division of the pollen grain nucleus takes place a short time before flowering, and always after the bulb is above the ground. Anthers may then be taken from the plant without destroying it, a thing unfortunately impossible at meiosis. The division does not take place simultaneously in all the pollen grains, nor is there any regular seriation as in the pollen mother cells. The time taken for all the pollen grains in one anther to divide is 6 or 7 days.

In the following list the dates of flowering are of course only approximate, since they vary considerably from year to year, and the period of flowering lasts 2 or 3 weeks. Nevertheless, they may be useful for comparative purposes. The species were grown in a cold greenhouse and the garden varieties out of doors. There is a difference of about 10 days in the time of flowering of the same variety under these different conditions.

IV. POLYPLOIDY AND CHROMOSOME MORPHOLOGY

The genus is divided systematically into two subsections, the *Eriostemon*es and the *Leiostemon*es, according to the presence or absence of hairs at the base of the filaments. Newton, whose work first showed the bearing of chromosome morphology and behaviour upon systematics in this genus, found that the groups thus formed differed also in the development of the embryo-sac. In the *Eriostemon*es the three successive divisions after meiosis are unpolarized, and the eight resulting

nuclei all lie at one end of the sac. In the *Leiostemones* the development corresponds to that found in *Lilium*.

TABLE I
Dates of division and of flowering (1933, 1934)

Garden varieties	Pollen mother cell meiosis	Pollen grain mitosis	Time of flowering
Diploids:			
Zulu	12 Sept.	—	5 May
Mrs Moon	23 "	16 Apr.	12 "
Louis XIV	30 "	—	6 "
Philippe de Commines }		31 Mar.	2 "
John Ruskin }		—	10 "
Inglescombe Scarlet	9 Oct.	—	4 "
Triploids:			
Keizerskroon	*2 Oct.	—	14 Apr.
Inglescombe Yellow	5 "	20 Apr.	14 May
Zomerschoon	5 "	—	15 "
Massenet	*8 "	27 Apr.	6 "
Cardinal Manning	c. 5 "	1 May	8 "
<i>Gesneriana spathulata</i>	9 "	23 Apr.	8 "
Pink Beauty	20 "	—	26 Apr.

With the exception of Inglescombe Scarlet, all the diploids go through meiosis earlier than the triploids.

Species			
<i>T. Eichleri</i>	c. 15 Oct.	21 Mar.	16 Apr.
<i>T. Fosteriana</i>	c. 15 "	12 Feb.	10 "
<i>T. primulina</i>	†22 "	—	17 "
<i>T. lanata</i>	c. 4 Nov.	27 Mar.	20 "
<i>T. praecox</i>	6 "	25 Feb.	11 "
<i>T. Kolpakowskiana</i>	8 "	20 Mar.	10 "
<i>T. galatica</i> × <i>armena</i> }	11 "	1 Apr.	3 May
<i>T. armena</i> × <i>galatica</i> }			
<i>T. armena</i>	27 "	2 "	1 "
<i>T. galatica</i>	—	6 "	3 "
<i>T. Korolkowi</i>	27 Nov.	—	24 Apr.
<i>T. turkestanica</i>	18 Dec.	27 Feb.	9 Mar.
<i>T. Whittalli</i>	18 "	29 Mar.	20 Apr.
<i>T. Orphanidea</i>	20 "	31 "	26 "
<i>T. saxatilis</i>	4 Feb.	29 "	20 "
<i>T. sylvestris</i>	c. 4 "	21 "	17 "
<i>T. Hageri</i>	17 "	9 Apr.	26 "
<i>T. stellata</i> , 4x	c. 15 "	18 Mar.	17 "
<i>T. Clusiana</i> , 5x	21 "	3 Apr.	18 "
<i>T. chrysantha</i> , 4x	10 Mar.	24 Mar.	18 "
<i>T. linifolia</i>	27 "	—	4 May

* Newton (1927).

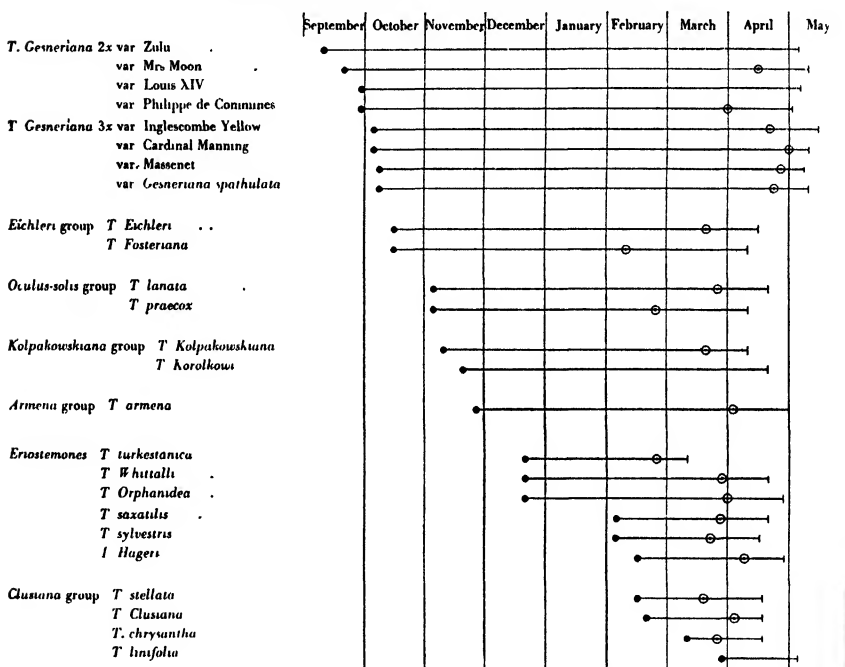
† This date, given by Newton, does not seem to fit in with those we have found for the other *Eriostemones*.

These two main groups, moreover, are mutually sterile. The *Leio-*
stemones are further divided into six subgroups which are distinguished

by morphological characters and in some cases, as we propose to show, by chromosome morphology.

These six groups are, so far as we know at present, inter-sterile, except as regards the garden tulips (*T. Gesneriana*). These are usually placed in the *Armena* group, but have failed to cross with *T. armena* and *T. galatica*. They have, however, produced three hybrids with the

TABLE II
Pollen development in species of Tulipa



The table shows the date of meiosis, the pollen grain divisions and the time of flowering in *Tulipa*. The species are arranged chronologically and fall by this method into the groups formed on systematic grounds. The line for each species begins at meiosis and ends at the time of flowering. The intervening mark represents the pollen grain division.

more distant *T. ingens* and *T. Griegi* of the *Eichleri* group. Two of these hybrids are completely male sterile. For this reason we have placed the garden tulips in a group of their own, but we do not propose to examine their position more closely at the present moment. It will become clear in the light of the genetical experiments to be described later.

In this connexion, however, we may mention that species placed together on systematic grounds go through meiosis on the male side at

approximately the same time, although they may flower at different times. The garden tulips, diploids first and then triploids, go through meiosis earliest and flower latest (see Table II). Next to these come the species in the *Eichleri* group, some of which have crossed with the garden varieties. The *Eriostemon*es form a distinct group of their own, although this comes in the middle of the various groups of *Leio*stemones. Latest of all comes the *Clusiana* group, which stands apart from all the others on grounds of gross structure, and also as we propose to show of chromosome morphology.

The haploid basic number in both groups is 12, and in both we find a similar range of polyploidy, that is to say, triploids, and tetraploids and in the *Leio*stemones one pentaploid. Polyploidy, especially triploidy, is common also among related genera and is presumably to be correlated with their method of reproduction. The diploid species of *Tulipa* probably reproduce mainly by seed, since they are commonly found in the wild as isolated bulbs dotted over a hillside. If the polyploids reproduced in the same way we should expect to find a number of aneuploid species, since they all produce gametes with different chromosome numbers, especially of course the odd-multiple polyploids, the triploids and pentaploids, where the range is considerable. No such aneuploids, however, occur. It is probable that such forms arose now and again when the polyploids set seed, but not being as vigorous as those they had to

TABLE III

Polyploidy in relation to the systematics of Tulipa. ($x = 12$)

A. ERIOSTEMONES

Diploid	Triploid	Tetraploid	Pentaploid
<i>T. australis</i> Link*	—	<i>T. sylvestris</i> L.*	—
<i>T. Griesbachii</i> Borbás	—	—	—
<i>T. persica</i> hort.	—	—	—
<i>T. primulina</i> Baker*	—	—	—
<i>T. urumiensis</i> Stapf	—	—	—
<i>T. Orphanidea</i> Boiss.*	—	<i>T. Whittallii</i> Elwes*	—
<i>T. Hageri</i> Held.*	—	—	—
<i>T. humilis</i> Herb.*	—	—	—
<i>T. pulchella</i> Fenzl*	—	—	—
<i>T. violacea</i> Boiss.	—	—	—
<i>T. Aucheriana</i> Baker	—	—	—
<i>T. cretica</i> Boiss.	<i>T. saxatilis</i> †	<i>T. saxatilis</i> (from Crete)	—
<i>T. biflora</i> Pall.*	—	<i>T. turkestanica</i> Regel*	—
<i>T. polychroma</i> Stapf	—	—	—
<i>T. tarda</i> Stapf*	—	—	—
(<i>dasytemon</i> Regel)			

Newton (1927).

† Darlington (unpublished).

B. LEIOSTEMONES

Diploid	Triploid	Tetraploid	Pentaploid
I. <i>Clusiana</i> group:			
<i>T. Clusiana</i> Dykes (Tibet)*	<i>T. Clusiana</i> (seedling, Merton)	<i>T. Clusiana</i> var. <i>Chitralensis</i> * <i>T. stellata</i> Hook. (several sources)† <i>T. chrysantha</i> Boiss.†	<i>T. Clusiana</i> DC.†
<i>T. chrysantha</i> (Kashmir)	—	—	—
<i>T. montana</i> Lindl. (<i>Wilsoniana</i> Hoog)	—	—	—
<i>T. linifolia</i> Regel†	—	—	—
<i>T. Batalini</i> Regel†	—	—	—
<i>T. Maximowiczii</i> Regel†	—	—	—
II. <i>Kolpakowskiana</i> group:			
<i>T. Kolpakowskiana</i> Regel†	—	<i>T. "Kolpakowskiana"</i> var. <i>coccinea</i> " (of commerce)	—
<i>T. Sprengeri</i> Baker†	—	—	—
<i>T. Korolkowi</i> Regel	—	<i>T. Korolkowi</i> var. <i>concolor</i>	—
<i>T. altaica</i> Pall.	—	—	—
<i>T. Ostrowskiana</i>	—	—	—
III. <i>Oculus-solis</i> group:			
<i>T. "cuspidata"</i> (van Tubergen)	<i>T. lanata</i> Regel	—	—
<i>T. kushkensis</i> Fedtsch.	—	—	—
<i>T. Schmidtii</i> Fomin	—	—	—
<i>T. oculus-solis</i> St Amans	<i>T. praecox</i> Tenore†	—	—
<i>T. Stapfii</i> Turrill	—	—	—
<i>T. cypria</i> Stapf	—	—	—
<i>T. undulatifolia</i> Boiss.	<i>T. aleppensis</i> Boiss. unnamed sp. D 50	—	—
<i>T. "Borsczowi"</i> (van Tubergen) (+ 0-6 ff.)	—	—	—
IV. <i>Armena</i> group:			
<i>T. suaveolens</i> Roth	—	—	—
<i>T. armena</i> Boiss.†	—	—	—
<i>T. galatica</i> Freyn (+ 2-12 ff.)	—	—	—
<i>T. planifolia</i> Jordan	—	—	—
<i>T. boeotica</i> Boiss.	—	—	—
<i>T. rhodopea</i>	—	—	—
<i>T. Gesneriana</i>	<i>T. Gesneriana</i>	—	—
V. <i>Eichleri</i> group:			
<i>T. Eichleri</i> Regel†	—	—	—
<i>T. Fosteriana</i> Hoog	—	—	—
<i>T. Micheliana</i> Hoog	—	—	—
<i>T. amabilis</i> Fedtsch.	—	—	—
<i>T. Griegi</i> Regel†	—	—	—
<i>T. Kaufmanniana</i> Regel†	—	—	—
<i>T. ingens</i> Hoog	—	—	—
<i>T. Tubergeniana</i> Hoog	—	—	—
<i>T. Hoogiana</i> Fedtsch.	—	—	—
<i>T. praestans</i> †	—	—	—
VI. <i>Edulis</i> group:			
—	—	<i>T. edulis</i>	—

* Darlington (1932).

† Newton (1927).

‡ Darlington (unpublished).

The identity of some of the above-mentioned species is somewhat doubtful. For example *T. "Kolpakowskiana* var. *coccinea*" is distributed commercially from Russia under that name. It is most probably a form of *T. Ostrowskiana*. Messrs C. G. van Tubergen's "*cuspidata*" consists of a mixed collection of bulbs, some of which are very probably *T. oculus-solis*. *T. "Borsczowi*" is also a commercial stock of uncertain identity.

compete with, were rapidly eliminated. We must conclude, therefore, that the odd-multiple polyploids and probably also the tetraploids are in effect clones, relying entirely upon asexual methods of propagation. These methods consist in the formation of offsets and droppers, and in the case of the more vigorous of long underground runners as well. The *Eriostemon*es in general possess great vegetative vigour, spreading very rapidly over a considerable area, but we find the habit also among the

TABLE IV

Polyploidy in T. Gesneriana, the garden tulip. (x = 12)

I. *Diploids*

Advance	Hybrid	La Reine*	Early
Alexandra	Dutch	La Tristesse	Darwin
Amiral de Constantinople	Parrot	Le Rêve	Early
Arethusa	Cottage	Louis XIV	Dutch
Bartigon	Darwin	"lutea major"	Parrot
Bleu Aimable	Darwin	Markgraaf van Baden	Parrot
Bouton d'Or	Cottage	Mayflower	Hybrid
Coffee Colour†	Parrot	Millet	Darwin
Crimson Beauty	Early	Mrs Hoog	Cottage
Duc van Thol, single*†	—	Mrs Moon	Cottage
Duc van Thol, Scarlet Duke†	—	Murillo†	Early
Duc van Thol, White Duke*	—	Perfecta	Parrot
Fantasy	Parrot	Philip de Commynes	Darwin
Fire King	Darwin	Pride of Haarlem†	Darwin
Gemma	Parrot	Proserpine*	Early
Inglescombe Scarlet	Cottage	"rubra major"†	Early
Isolda	Cottage	Suzon	Darwin
John Ruskin	Cottage	Tournesol*	Early
La Candeur*	Darwin	White Swan	Early
La Merveille	Cottage	Zulu	Darwin

II. *Triploids*

Cardinal Manning (or Goliath)*	Cottage
"Gesneriana spathulata"	Cottage
Keizerskroon†	Early
Inglescombe Yellow	Cottage
Massenet†	Darwin
Pink Beauty*	Early
Zomerschoon	Cottage

* de Mol (1928).

† Newton (1927).

Leiostemones, particularly among the triploids. This is not characteristic of all triploids, since some of the garden varieties increase very slowly. It is probable therefore that when a triploid was formed possessing this power of rapid vegetative reproduction, it survived and established itself. Only in the more sheltered conditions of the garden have less vigorous triploids been preserved.

The polyploids in the first group have different specific names from the diploids from which they have presumably been derived (Table III A). From

the cytological as well as the morphological point of view, however, there seems to be little or no difference between such specific polyploids and polyploids which are described as forms of diploid species. The differences, of which size is the most obvious, are of the same order. We may say, for example, that *T. sylvestris* is probably the tetraploid form of *T. australis*, *T. Whittalli* of *T. Orphanidea*, and *T. turkestanica* of *T. biflora*. With rather less certainty we may also describe *T. saxatilis* as the triploid form of *T. cretica*. This species is the only triploid in the group and is almost completely sterile, at least on the male side. It propagates very rapidly by means of the runners characteristic of the Eriostemones. This



Fig. 1. *T. saxatilis*. $2n=36$.



Fig. 2. *T. saxatilis*. $2n=48$.

Figs. 1 and 2. Triploid and tetraploid forms of *T. saxatilis* (Eriostemones) ($\times 2000$). (Fig. 1 by permission of Dr C. D. Darlington.)

year (1935) a new form sent in by Mr G. P. Baker was found to be tetraploid (Figs. 1 and 2). From the high proportion of bad pollen, it is probably an autotetraploid just as the commoner form is an autotriploid.

Among the Leioestemones (Table III B), *T. clusiana* contains the widest range of polyploid forms. The common form of this delicate pink and white tulip grows along the coasts of the Mediterranean and in Spain. Newton reported it to be pentaploid with 60 chromosomes (Fig. 6). Later other varieties were examined, including one from Chitral and a single isolated bulb from Tibet. Darlington (1932 a, p. 210) illustrates these forms, which proved to be tetraploid and diploid respectively (Figs. 3 and 5). The tetraploid is similar to the pentaploid in size and form, but the

diploid is much smaller and has wrinkled edges to the leaves, and a very short flower-stalk in marked contrast to the tall habit of the other two. This plant, which is normally self-sterile, set natural seed in 1931. It was thought that it might have been accidentally crossed with a neighbouring tetraploid, which was in flower at the same time. About sixty



Fig. 3. $2n=24$.



Fig. 4. $2n=36$.



Fig. 5. $2n=48$.



Fig. 6. $2n=60$.

Figs. 3-6. Small chromosome type ($\times 2000$). Forms of *T. Clusiana*. (Figs. 3 and 5 from Darlington, 1932.) The triploid form has two chromosomes with secondary constrictions (S) close to the centric constriction, in the long arm.

plants were obtained from this capsule, of which eleven were examined cytologically. They were all triploid (Fig. 4). In view of the behaviour of the tetraploid, which produces gametes of widely different chromosome number, the original explanation seems unlikely. It is more probable that the family arose by failure of reduction of the ovules followed by selfing. Newton points out that doubling in the embryo-sac at the

chalazal end in the second division of meiosis is quite common among the *Leiostemones*. On two occasions in *T. Kolpakowskiana* he observed its occurrence at the micropylar end. This, followed by fertilization with haploid pollen, would give triploid offspring. It is remarkable, nevertheless, that such doubling occurred, as we must suppose, in every fertilized ovule in the capsule. It is probable that this behaviour accounts for the commonness of autotriploids among the tulips, and indeed among other related genera such as *Fritillaria* and *Lilium* (Newton & Darlington, 1929).

In no other species except *Crepis capillaris* (Nawaschin, 1926) and *Prunus spinosa* (Mather, 1936; Fabergé, unpublished) has such a range

TABLE V
Chromosome types in Tulipa

<i>Small</i> Short arms v. small	<i>Medium</i> Short arms small	<i>Large</i> Short arms small, or large in some complements	<i>Atypical</i>
<i>Clusiana</i> group: <i>T. Clusiana</i> 2x, 3x, 4x and 5x <i>T. chrysantha</i> 2x and 4x <i>T. stellata</i> , 4x <i>T. linifolia</i> , 2x <i>T. Batalini</i> 2x <i>T. Maximowiczii</i> 2x <i>T. montana</i> (<i>Wilsoniana</i>) 2x	<i>Kolpakowskiana</i> group: <i>T. Korolkowi</i> 2x and 4x <i>T. lanata</i> 3x <i>T. Kolpakowskiana</i> 2x and 4x <i>T. altaica</i> 2x <i>Oculus-solis</i> group: <i>T. cuspidata</i> 2x <i>T. kuschkinsis</i> 2x	All other <i>Leiostemones</i> , including <i>T. Gesneriana</i> All <i>Eriostemones</i>	<i>T. Schmidtii</i> 2x Species with new chromosome types resulting from structural change <i>T. galatica</i> 2x + ff. <i>T. Borsczowi</i> 2x + ff. <i>T. Gesneriana</i> var. Mrs Moon 2x var. Zomerschoon 3x

of polyploidy been found. No other pentaploid form is known in the genus. This is probably correlated with the smaller size of the chromosomes of *T. Clusiana*. Newton divided the genus into three groups according to the types of chromosome complement. *T. Clusiana* comes in the first group, which has relatively small slender chromosomes with subterminal centric constrictions. The short arms are all very small, and in many cases spherical. To this group belong also those species placed nearest to *T. Clusiana* on systematic grounds, *T. stellata*, *T. chrysantha*, *T. linifolia*, *T. Batalini* and others (Table V).

The second group has chromosomes slightly larger than *T. Clusiana*, with the short arms variable in length. Newton placed *T. Kolpakowskiana* in this group, and further investigation has added other species, for

example *T. altaica*, *T. Korolkowi* and *T. lanata* (Figs. 9–13), together with the unrelated species *T. cuspidata* and *T. kuschkensis*.

The third group has large thick chromosomes with submedian or subterminal centric constrictions. This is the largest group and contains all the Eriostemones, and the remaining Leiostemones, including the garden varieties. *T. coccinea* (Fig. 14) is an example of a diploid species, and *T. praecox* (Fig. 15) and *T. edulis* (Fig. 16) of polyploids.

In the small and medium group we find nearly all the polyploids. In the first group we have the four forms of *T. Clusiana* and two other tetraploids, *T. stellata* and *T. chrysantha*. This last is usually tetraploid, but has a dwarf diploid form resembling the diploid *T. Clusiana* in appearance (Fig. 7).

In the medium group we find a tetraploid form of *T. Kolpakowskiana* (Fig. 13), which has always been regarded as entirely diploid, *T. Korolkowi*, which also has a diploid form (Figs. 9, 10) and *T. lanata* (Fig. 12).



Fig. 7. *T. chrysantha*. $2n=24$.



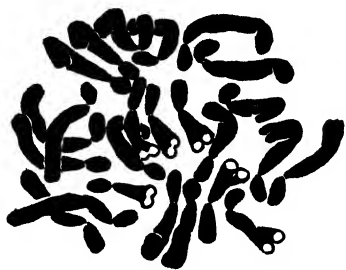
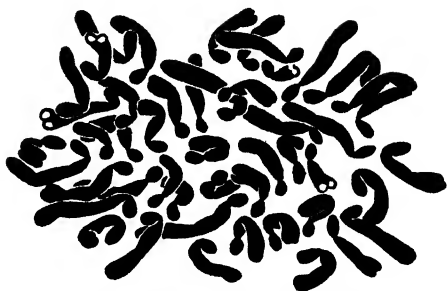
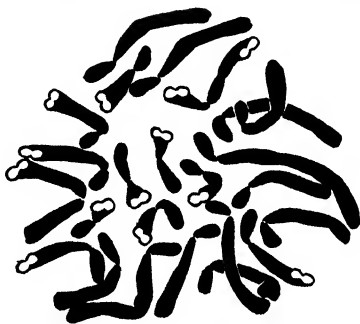
Fig. 8. *T. Wilsoniana*. $2n=24$.

Figs. 7 and 8. Small chromosome group ($\times 2000$). These two species each have a pair of chromosomes with secondary constrictions (S) in the short arm.

The only two polyploid species in the large group are *T. praecox* and *T. edulis*. The garden varieties, however, which include several triploids, but somewhat surprisingly no tetraploids (Table IV), also belong to this group.

It would appear therefore among the wild Leiostemones that polyploids can survive more easily amongst species with small or medium chromosomes. The Eriostemones and the garden varieties on the other hand seem to be able to accommodate a greater mass of chromosome material, possibly owing to an increase in cell size.

In addition to the three groups described by Newton, further investigation has revealed the occurrence of an atypical species which cannot be placed in any group. This species, *T. Schmidtii* (Fig. 17), has chromosomes unlike those of any other member of the genus. Although long and slender like those of the first group, they have median and submedian

Fig. 9. *T. Korolkowi*. $2n=24$.Fig. 10. *T. Korolkowi*. $2n=48$.Fig. 11. *T. Kuschkenssis*. $2n=24$.Fig. 12. *T. lanata*. $2n=36$.Fig. 13. *T. Kolpakowskiana*. $2n=48$.Figs. 9-13. Medium chromosome group ($\times 2000$).

centric constrictions like those of the third. They also possess secondary constrictions and trabants and are in general appearance more like the chromosomes of *Fritillaria* than those of *Tulipa*.



Fig. 14. *T. coccinea*. $2n=24$.



Fig. 15. *T. praecox*. $2n=36$.

Fig. 16. *T. edulis*. $2n=48$.

Figs. 14-16. Large chromosome group ($\times 2000$). *T. coccinea* has a pair of chromosomes with trabants. They appear to be attached to the end of the spiral thread, as in *Tradescantia* (Darlington, 1929).

The changes of size and shape so far described affect the whole complement. There have also been a large number of structural changes affecting individual chromosomes. Only a few of these, such as reduplications or large translocations, can be recognized at mitosis. *T. galatica* and *T. Borsczowi*, for example, possess, in addition to the normal complement, a variable number of short chromosomes not represented elsewhere

in the genus. These have been found by Darlington (unpublished) to behave as reduplicated fragments at meiosis. *T. galatica* and some of the garden varieties possess odd chromosomes for which no exactly similar mate exists. The most extreme example of this is found in the old garden

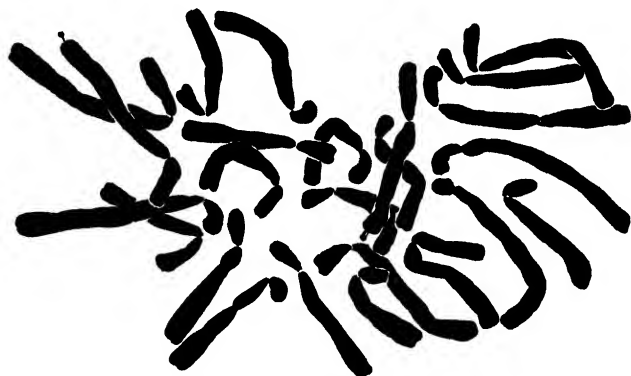


Fig. 17. *T. Schmidtii*. $2n=24$.



Fig. 18. Garden variety "Zomerschoon". $2n=36$.

Figs. 17 and 18. Atypical species ($\times 2000$). *M*, chromosome with median constriction. *T*, chromosome with terminal constriction.

tulip Zomerschoon. This is a triploid and has been in cultivation since the seventeenth century. In the somatic complement of this clone are two chromosomes of a kind common in other genera (*Lilium*, *Fritillaria*) but unknown elsewhere in *Tulipa*. One has a nearly terminal centromere, the short arm being reduced to a minute sphere of diameter less than half that of the rest of the complement. The other is a long chromosome

whose centromere is nearly median (Fig. 18, *M*, *T*). The possibility of a translocation between two originally equal and homologous arms immediately suggests itself, but the evidence for such changes must be considered in relation to meiosis. This variety produces very little good pollen. It is possible that this and its heterozygous chromosome types may be due to an accumulation of structural changes which have occurred during its 400 years of clonal life.

V. CHROMOSOME MORPHOLOGY AND SYSTEMATICS

The value of chromosome morphology as a means of determining relationships is clearly limited by the fact that each complement is subject to its own genotypic control (Darlington, 1932 *b*). We find in the present genus that changes of size have occurred within the *Leiostemones*, but not between the *Leiostemones* and *Eriostemones*. Since the species in the first group are all closely related, it is probable that the same series of changes isolated them from the rest of the genus. Parallel but independent changes must have produced the second group, since it contains species not regarded as closely related by systematists. *T. Kolpakowskiana* for example is placed in a different group from *T. cuspidata*, which is apparently more closely related to *T. oculus-solis* and other species with large chromosomes. A further independent change must have isolated *T. Schmidtii*, which also belongs morphologically to this group.

The differences between these four types of chromosome complement can be attributed to genotypical and structural changes. The first control those changes affecting size and degree of spiralization which all chromosomes undergo together. The second determine those of which the most obvious at mitosis are the position of the centromere, and the relative length of the arms.

But beyond this first analysis, we see that there is a uniformity in the position of the centromere throughout the complement which makes it possible to distinguish these types, and this uniformity implies something more than random structural change—it implies a regulation. This regulation, although not directly genotypic, must depend ultimately on genotypically controlled conditions to which it is an adaptation. What these conditions are may be seen from a consideration of the arrangement of the chromosomes on the metaphase plate at mitosis and their separation at the first anaphase of meiosis. If the size of the spindle is small, single-armed chromosomes take up less space on the plate and allow therefore of regular congression. If chiasmata are numerous and

un-terminalized, the separation of the bivalents at anaphase will be more regular if they are uniformly one- or two-armed. Thus a uniformity in the complement may be indirectly imposed as an adaptation to mechanical conditions of mitosis and meiosis. In a word, these conditions may be held responsible for the typical patterns of chromosomes which distinguish systematic groups.

VI. SUMMARY

1. The basic haploid chromosome number in the genus *Tulipa* is 12. Polyploid series occur in both the subsections Eriostemones and Leio-stemones.

2. Although the diploid species probably reproduce sexually in the wild, the polyploids must be purely clonal since no aneuploids have been found.

3. Two main types of change have affected the chromosome morphology of the genus. Genotypic change of size and regulated structural change altering the position of the centromere have separated the genus into three groups with large, medium and small chromosomes. Random structural change has produced complements with reduplicated fragments (*T. galatica*) and with clearly unmated chromosomes (*T. Gesneriana* var. *Zomerschoon*).

4. Evidence from external and chromosome morphology, from inter-sterility and from the time at which meiosis occurs, points to the conclusion that the subgroup *Clusiana* of the Leio-stemones is as distinct from the other subgroups as it is from the Eriostemones.

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FURTHER LINKAGE DATA ON THE ALBINO CHROMOSOME OF THE HOUSE-MOUSE

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INTRODUCTION

In a previous paper (Grüneberg, 1935) the author described a three-point back-cross experiment in the house-mouse involving the linked factors for shaker₁, albinism and pink-eye dilution. It was shown that the three genes are arranged in that order. The presence of interference was shown to be likely, but the total of 1144 animals was too small to allow for a definite answer. On the other hand, the figures, so far as they went, seemed to indicate that interference, if present, was less strong than in *Drosophila*. No definite answer could be given as to whether the cross-over values vary with the age of the parents.

To deal with these open questions, the experiments were continued on a larger scale. The total (including the published data) has now been brought up to 3692 animals. An analysis of all these data will be given in this paper. In view of some unexpected differences between the batches in which the experiments were performed, a more detailed description of the way in which the experiments were prepared seems necessary.

THE EXPERIMENTS

In 1933 a number of pink-eyed shaker mice were given to us by Dr W. H. Gates for further studies. One of the original males (No. Z1) when out-crossed to an extreme dilute ($c^p c^D$) female produced 10 wild type and 9 black-eyed white offspring. That animal was therefore heterozygous for albinism. The black-eyed whites, being of the constitution $\frac{sh\ c\ p}{+ c^D +}$, were inbred. In the F_2 generation, albino shakers were produced. Since in the presence of albinism pink-eye dilution cannot be scored, these animals were tested individually by crossing to pink-eyed (pp) mice. Males found to be of the constitution $\frac{sh\ c\ p}{sh\ c\ p}$ were used as the triple recessives in the back-cross published (Exp. I). It proved

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difficult to establish a pure stock of these animals, since shaker females in the early stages of the experiment were very bad mothers. Later, this has changed fundamentally, and in the last experiments shaker females have proved excellent mothers, the average litter size at recording age being 7.4 young; in one case, as many as 14 young have been born and raised by a shaker mother.

The unsatisfactory behaviour of shaker females at the beginning made an out-cross necessary. One of the original pink-eyed shaker males was therefore out-crossed to commercial females of the constitutions **aaBBDdss** and **aabbddss**. Animals extracted in F_2 and back-crosses have served as a supply of pink-eyed shakers. In later stages of the work the factors **a**, **b**, and **d** have segregated in a number of matings.

The majority of triply heterozygous $\left(\frac{\text{sh} + \text{p}}{+ \text{c}^{\text{D}} +}\right)$ females for Exp. I were secured by crossing extracted **sh p.sh p** males to **c^Dc^D** females. In addition, the few wild-type daughters of Z 1 ♂ were used.

Exp. II was done on a small scale only, since at that time the shaker females were still rather unreliable. The type of cross was

$$\frac{\text{sh} + \text{p}}{+ \text{c}^{\text{D}} +} \delta \times \frac{\text{sh c p}}{\text{sh c p}} \text{♀},$$

that is, the reciprocal experiment to Exp. I. The animals were derived from the same sources as in that experiment.

In Exp. I two double cross-overs of the constitution $\frac{\text{sh c}^{\text{D}} \text{p}}{\text{sh c p}}$ were obtained. One of them, a female, forms the basis of all further experiments. She was out-crossed to an extreme dilute male and produced 16 black-eyed white and 17 extreme dilute offspring. The former were discarded. By inbreeding the extreme dilutes, mice of the constitution $\frac{\text{sh c}^{\text{D}} \text{p}}{\text{sh c}^{\text{D}} \text{p}}$ were obtained. Males of this stock served as triple recessives in Exp. III. The triple heterozygotes for this experiment were obtained by crossing a $\frac{\text{sh c p}}{\text{sh c p}} \delta$ previously used in Exp. I to females of a Black-agouti (**ABD**) pure line. Exp. III was therefore of the type

$$\frac{\text{sh c}^{\text{D}} \text{p}}{\text{sh c}^{\text{D}} \text{p}} \delta \times \frac{\text{sh c p}}{+ + +} \text{♀},$$

the females being the heterozygous sex as in Exp. I, but all three factors were in coupling.

Non-cross-overs obtained from Exp. III were used for Exps. IV and V. The constitutions of the animals were

$$\frac{\text{sh } c^D \text{ p}}{\text{sh } c \text{ p}} \sigma \times \frac{\text{sh } c^D \text{ p}}{+ + +} \varphi \quad (\text{Exp. IV})$$

and

$$\frac{\text{sh } c^D \text{ p}}{+ + +} \sigma \times \frac{\text{sh } c^D \text{ p}}{\text{sh } c \text{ p}} \varphi \quad (\text{Exp. V})$$

respectively. As is obvious, $c^D c^D$ and $c^D c$ young will be expected in equal numbers in animals not carrying the wild-type allelomorph of albinism. In the presence of pp these types are indistinguishable. Only in cross-overs between c and p does the appearance of two different classes become noticeable. Since the discrimination between the two types in the baby coat is sometimes difficult, these two classes are combined in the following tables. Moreover, in the presence of bb or dd which segregated in some of the matings, the distinction between these types is very unreliable.

In a few cases in Exp. IV males of the $\frac{\text{sh } c^D \text{ p}}{\text{sh } c^D \text{ p}}$ stock were used as triple recessives. In such matings all the animals in question are $c^D c^D$.

RESULTS

The results of these experiments, including the data already published, are given in Tables I and II. For comparison of the relative viabilities of corresponding classes, the totals of Exps. I and II and of Exps. III, IV and V are given. As is obvious from the χ^2 's, in none of the cases is there a serious disturbance of the ratios, at least not in the totals. We shall have to come back to that point later. The single-factor ratios in the experiments are given in Table III. They, too, do not show any disturbance in the grand totals, though a closer analysis tells a different story.

The linkage values obtained in the five experiments are given in Table IV. That table also gives the number of double cross-overs observed and the number expected on the assumption that there is no interference. As is seen, altogether 21 double cross-over individuals should have occurred on that assumption, whereas only 5 were actually found.

Arranging the whole of the data in an ordinary fourfold table leads to $\chi^2 = 14.439$ for one degree of freedom, while Yates' correction for continuity lowers the value slightly to 13.714. The odds against that happening due to chance alone are about 1:5000. The existence of

TABLE I

			$\frac{sh + p}{+ c^D +} \times \frac{sh c p}{sh c p}$			
Experiment	I	II	Total	χ^2
Sex of heterozygous parent ...			♀	♂		
0 pink-eyed shakers			464	135	599	2.589
0 black-eyed whites			484	172	656	
1 black-eyed white shakers			16	2	18	0.400
1 pink-eyed			18	4	22	
2 black-eyed shakers			85	23	108	0.391
2 pink-eyed whites			75	24	99	
1, 2 pink-eyed white shakers			2	—	2	—
1, 2 wild type			—	—	—	
Total			1144	360	1504	

TABLE II

			$\frac{sh c p}{+ + +} \times \frac{sh c^D p}{sh c^D p} *$				
Experiment	III	IV	V	Total	χ^2
Sex of heterozygous parent ...			♀	♀	♂		
0 pink-eyed white shakers			355	260	265	880	0.005
0 wild type			411	239	233	883	
1 black-eyed shakers			21	13	10	44	0.835
1 pink-eyed whites			25	15	13	53	
2 { black-eyed white } shakers			72	43	31	146	3.351
2 { extreme dilute }			93	52	34	179	
1, 2 pink-eyed shakers			—	—	—	—	—
1, 2 { black-eyed whites } extreme dilutes			1	2	—	3	
Total			978	624	586	2188	

* For the differences between the experiments see text.

TABLE III

Single-factor ratios

Experiment ...	I	II	III	IV	V	Total	Homo- geneity test $\chi^2 (n=4)$
Sh	577	199	530	308	280	1894	9.907
sh	567	161	448	316	306	1798	
$\chi^2 (n=1)$	0.087	4.011	6.875	0.103	1.154	2.496	10.561
C	567	163	525	304	277	1836	
c (c^D)	577	197	453	320	309	1856	8.229
$\chi^2 (n=1)$	0.087	3.211	5.301	0.410	1.747	0.108	
P	585	196	505	297	274	1857	8.229
p	559	164	473	327	312	1835	
$\chi^2 (n=1)$	0.591	2.872	1.047	1.442	2.464	0.131	

For 4 degrees of freedom $P_{0.10}=7.779$; $P_{0.05}=9.488$; $P_{0.01}=11.668$.

interference in the mouse can therefore be regarded as being established beyond doubt.

On the other hand, it is not so certain whether that interference is in fact weaker than in *Drosophila*. As pointed out previously, in a similar case in that organism, we should expect a coincidence of 0.1 or less. This would lead to an expectation of 2.1 or less double cross-over individuals, whereas 5 were actually found.

TABLE IV

Experiment	I	II	III	IV	V	Total
Sex of heterozygous parent ...	♀	♂	♀	♀	♂	
Cross-over percentage:						
sh-c	3.1	1.7	4.8	4.8	3.9	3.8
c-p	14.2	13.1	17.0	15.5	11.1	14.5
Double cross-over individuals:						
Expected	5.1	0.8	8.0	4.6	2.5	21.0
Observed	2	—	1	2	—	5

Now, when the frequency of a type in a population is q , the probability that 5 or more will be found in a sample of n individuals is P , where

$$1 - P = (1 - q)^n + nq(1 - q)^{n-1} + \frac{n(n-1)}{2!} q^2(1 - q)^{n-2} \\ + \frac{n(n-1)(n-2)}{3!} q^3(1 - q)^{n-3} + \frac{n(n-1)(n-2)(n-3)}{4!} q^4(1 - q)^{n-4}.$$

In our case, $n=3692$, $q=0.0005688$. So $P=0.0577$. It is, therefore, likely but not certain that interference is weaker than in *Drosophila*.

Comparing the cross-over values obtained for females and males, there is a lower value for males in both sections when I is compared with II, or III and IV are compared with V. This corresponds well to the expectation.

What does not correspond to expectation is that the value for sh-c in V (♂♂) is higher than that in I (♀♀). The difference is not significant, but is in the wrong direction. As is obvious from Table IV, for the sh-c interval the values of III and IV are distinctly higher than that for I, while V is markedly higher than II. In other words, for the sh-c section the values obtained from coupling are consistently higher than those obtained from repulsion in both sexes. Significance tests by means of fourfold tables lead to $\chi^2=4.659$ in the case of the females and to $\chi^2=3.827$ in the case of the males ($n=1$). Adopting the 5 per cent. level of significance, both differences are to be regarded as established. The

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evidence from both sexes may be combined by adding the respective χ^2 's and ascribing to this sum the standard error $\sqrt{2}$. Then we get

$$2.158 + 1.956 = 4.114 \pm 1.414.$$

The odds against so big a deviation being due to chance alone are less than 1 : 250. There can, therefore, be little doubt that these differences are significant, whatever the reason may be.

Now, turning to the **c-p** section the situation is less clear. The coupling data in females are higher than the repulsion data, thus showing the same trend as the **sh-c** interval. Comparison of I with III+IV leads to $\chi^2 = 2.597$ for one degree of freedom which, though it is not significant in itself, is in the "right" direction. In males the (not significant) difference goes in the "wrong" direction; however, that value by itself does not exclude the possibility that the expected difference is obscured by random sampling.

This curious and unexpected situation led to a careful reinvestigation of all the data published about the albino chromosome of the mouse. A complete list of the papers referred to below has been given previously (Grüneberg, 1935).

In the **sh-c** interval Gates' figures (1931) show the same trend as my own data, though to a slightly lesser extent. Comparing coupling and repulsion in females (combined data) leads to $\chi^2 = 3.600$ for one degree of freedom which in itself is not significant though suspiciously high. For males we get by the same method $\chi^2 = 3.290$, the deviation being in the same direction. Combining the evidence from both sexes as above, the sum of χ^2 's is 3.711 ± 1.414 . This corresponds to $P = 0.01$ or, in other words, a difference as big or bigger than that observed due to chance alone would occur only once in 100 trials.

TABLE V
sh-c interval

Author	Coupling						Repulsion					
	♀♀			♂♂			♀♀			♂♂		
	Cross-overs			Cross-overs			Cross-overs			Cross-overs		
	<i>n</i>		%	<i>n</i>		%	<i>n</i>		%	<i>n</i>		%
Gates (1931)	1920	79	4.1	240	7	2.9						
Grüneberg	1602	77	4.8	586	23	3.9	1144	36	3.1	360	6	1.7
(all data)												
Total	3522	156	4.43	826	30	3.63	1144	36	3.15	360	6	1.67

The calculations for the **c-p** section are complicated by the fact that in the earlier experiments of Dunn (1920), Castle & Wachter (1924) and

the coupling data of Detlefsen & Clemente (1924), the bottom recessive *c* was used. The young which were classified immediately after birth fell into two classes only, viz. black-eyed and pink-eyed individuals. In repulsion ($\frac{cP}{Cp} \times \frac{cP}{cP}$) the dark-eyed young recovered represent one-half of the cross-overs, whereas the corresponding class and both non-cross-over types are pink-eyed. The cross-over percentage is therefore esti-

TABLE VI

c-p interval. Coupling. Females

Type of data	Author	<i>n</i>	Smaller class	Estimate of <i>p</i>	Amount of information
a	Feldman (1924)	1040	208	0.2000	
a	Gruneberg (new data)	1602	263	0.1642	
a	Total	2642	471	0.1783	18,035
c	Dunn (1920)	1420	595	0.1620	
c	Castle & Wachter (1924)	556	225	0.1906	
c	Detlefsen & Clemente (1924)	439	194	0.1162	
c	Total	2415	1014	0.1602	2,479
a + c	Total	—	—	0.1761	20,514

Cross-over value = 17.61 ± 0.698 %.

TABLE VII

c-p interval. Repulsion. Females

Type of data	Author	<i>n</i>	Smaller class	Estimate of <i>p</i>	Amount of information
a	Detlefsen & Clemente (1924)	98	20	0.2041	
a	Feldman (1924)	313	51	0.1629	
a	Gruneberg (1935)	1144	162	0.1416	
a	Total	1555	233	0.1498	12,207
b	Dunn (1920)	1369	107	0.1563	4,750
a + b	Total	—	—	0.1516	16,957

Cross-over value = 15.16 ± 0.768 %.

Difference between coupling and repulsion in females 2.45 ± 1.038 %.

$D/m = 2.360$, $P = 0.02$.

mated by doubling the number of dark-eyed young and calculating the cross-over value on this basis.

In coupling ($\frac{CP}{cP} \times \frac{cP}{cP}$) the dark-eyed young represent one non-cross-over class, while the other non-cross-over class and both cross-over types show pink eyes. The cross-over percentage is therefore estimated by using the excess of pink-eyed over black-eyed young as a basis.

In the repulsion experiment of Detlefsen & Clemente (1924) and all the experiments of Feldman (1924) and myself, all four possible classes are directly recognizable.

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Evidently, the amount of information furnished by the earlier experiments is much less than that of the later ones. Let $100x$ be the cross-over value of **c-p**. Then we have estimates of

- (a) x from complete back-cross data,
- (b) $\frac{x}{2}$ from incomplete repulsion data,
- (c) $\frac{1-x}{2}$ from incomplete coupling data.

In the latter two cases the amount of information (I) about x is $\frac{1}{4}$ that about $\frac{x}{2}$ or $\frac{1-x}{2}$, i.e. $\frac{(a+b)^3}{4ab}$, where a and b are the numbers in the two classes.

TABLE VIII
c-p interval. Coupling. Males

Type of data	Author	n	Smaller class	Estimate of p	Amount of information
a	Feldman (1924)	129	15	0.1164	
a	Grüneberg (new data)	586	65	0.1109	
a	Total	715	80	0.1119	7,196
c	Dunn (1920)	1911	819	0.1429	
c	Castle & Wachter (1924)	3324	1431	0.1390	
c	Detlefsen & Clemente (1924)	698	329	0.0573	
c	Total	5933	2579	0.1306	6,036
a + c	Total	—	—	0.1204	13,232

Cross-over value = 12.04 ± 0.869 %.

TABLE IX
c-p interval. Repulsion. Males

Type of data	Author	n	Smaller class	Estimate of p	Amount of information
a	Detlefsen & Clemente (1924)	376	45	0.1197	
a	Feldman (1924)	275	32	0.1164	
a	Grüneberg (new data)	360	47	0.1306	
a	Total	1011	124	0.1227	9,395
b	Dunn (1920)	1772	115	0.1298	7,300
a + b	Total	—	—	0.1258	16,695

Cross-over value = 12.58 ± 0.774 %.

Difference between coupling and repulsion in males -0.54 ± 1.16 %.

$D/m = 0.464$. $P = 0.62$.

The result of these calculations is given in Tables VI-IX. In females, crossing-over in the **c-p** interval is higher in coupling (17.61 ± 0.698 per cent.) than in repulsion (15.16 ± 0.768 per cent.). The difference of these values is 2.45 ± 1.038 per cent., and therefore 2.36 times its standard error. The odds against so big a deviation being due to chance alone are

1:50. In males there is still a very slight and insignificant deviation in the wrong direction. (Coupling 12.04 ± 0.869 per cent.; repulsion 12.58 ± 0.774 per cent. Difference -0.54 ± 1.16 per cent. $D/m = 0.464$. $P = 0.62$.)

The whole of the evidence for both chromosome regions and both sexes is summarized in Table X, where, for sake of convenience, everything is expressed in terms of χ . I am inclined to interpret this table

TABLE X
Difference between coupling and repulsion. χ table

	♀♀	♂♂	Both sexes	P
sh-c	+1.897	+1.814	+3.711	0.01
c-p	+2.360	-0.464	+1.896	0.18
Both intervals	+4.257	+1.350	+5.607	
P	0.0026	0.34	—	0.0052

thus. There is strong evidence that in females crossing-over is higher in coupling than in repulsion in both marked intervals, the sum of the χ 's being as high as 4.257 ± 1.414 , corresponding to $P = 0.0026$. It seems doubtful whether a similar effect exists in males, although there is some indication of it in the sh-c interval, for which the sum of χ 's for both sexes is 3.711 ± 1.414 ($P = 0.01$).

We are left to decide whether in the females the 1 chance in 385 has been a reality in the work with the albino chromosome; or else we must invent an *ad hoc* hypothesis to explain the apparent inconsistency of the data with the classical theory which postulates the same linkage values for coupling and repulsion. The author is inclined to suspend judgement of the matter for the time being, since he is unable to present any plausible hypothesis.

Crossing-over and age of parents

All the data were investigated carefully as to whether there is any change of crossing-over frequency with the age of the parents. The material was subdivided in five age groups (2-4, 5-6, 7-10, 11-14, and over 14 months), the last group being represented by small numbers only. Each experiment was treated separately. The result was completely negative. The values showed a random distribution without any indication of consistent changes with age. It seems unnecessary to give the whole of this negative evidence in detail.

Inhomogeneity of data

As mentioned above, the single-factor ratios in the grand totals (Table III) do not show a serious deviation from the expected equality of corresponding allelomorphs. However, the deviations in the single

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experiments are markedly greater than expected on the basis of random sampling alone. The sets of data were therefore tested for homogeneity by means of $2 \times n$ tables. This leads to values of χ^2 (for 4 degrees of freedom) of 9.907 for **Sh/sh**, 10.561 for **C/c** and 8.229 for **P/p**.

For 4 degrees of freedom, the chance of a deviation greater than 7.779 is 0.10, of a deviation greater than 9.488 is 0.05 and of a deviation greater than 11.668 is 0.02. Adopting the 5 per cent. level of significance, two out of the three values are to be regarded as significant, while the third one is suspiciously high. The figures, therefore, suggest that the data are probably inhomogeneous, and that causes other than random sampling are responsible for the excess of deviation. Since all the animals were bred under the same conditions, and in Exps. III-IV even largely simultaneously, it seems unlikely that exogenous causes are responsible for this apparent inhomogeneity. The alternative is the assumption of genetical causes. As a suggestion the hypothesis may be put forward that a lethal factor linked with the genes for shaking, albinism and pink-eye dilution segregated in some of the pairs. In coupling with the recessives, such a factor would lead to an excess of the dominants in those matings in which it segregates, while in repulsion an excess of recessives would result. If such a factor is present in coupling and repulsion in about the same frequency, its effect would be blotted out in the grand totals. The material was sufficiently inbred during the experiments to make such a segregation possible.

The investigation of the material according to single paired matings does not allow for a definite decision, since many of the families are too small to be of much value for this question. On the whole the deviations of single families from the total of an experiment are greater than would be expected from chance alone, but this excess is not significant in our material which was not bred for the solution of this question. It seems unprofitable at this stage to discuss all the pros and cons in detail, since new experiments are being undertaken which, it is hoped, will give a firmer basis for such a discussion.

Whatever the result of these experiments may be, it seems clear that even the presence of linked lethals would not account for the difference of crossing-over in coupling and repulsion. In the first place, the good fit in the grand totals of Table III shows that a lethal, if present, was sufficiently evenly distributed over coupling and repulsion as not to influence the cross-over values. Secondly, it seems hard to believe that such a lethal (or several) were present in the experiments of the other authors whose experimental data show the same trend.

SUMMARY

1. A trifactorial back-cross in the house-mouse involving the genes for shaker₁, albinism and pink-eye dilution was carried out reciprocally in alternating repulsion and in coupling. Altogether 3692 animals were bred.

2. The existence of interference in the sections marked was conclusively proved. It is likely, though by no means certain, that this interference is weaker than in *Drosophila*.

3. Crossing-over seems to be more frequent in coupling than in repulsion in both marked intervals in females and possibly in males. The same trend is found in the data of previous investigators who worked with this chromosome. No plausible explanation for this unexpected difference could be given.

4. Combining the data from the literature and those presented in this paper leads to the following weighted mean cross-over values:

	Females		Males	
	Coupling	Repulsion	Coupling	Repulsion
sh-c	4.43 ± 0.35	3.13 ± 0.52	3.63 ± 0.65	1.67 ± 0.68
c-p	17.61 ± 0.70	15.16 ± 0.77	12.04 ± 0.87	12.58 ± 0.77

5. No change of crossing-over with the age of the parents was found.

6. Inhomogeneity in the data as regards the single-factor ratios is shown to exist. It is suggested that this may be due to a linked lethal factor segregating sometimes in coupling, sometimes in repulsion.

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A STUDY OF MUTATIONS IN EVOLUTION.

III. THE EVOLUTION OF THE EQUINE FOOT

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(With Two Text-figures)

THE development of the extremities of the horse does not comply in all respects with the simple generalizations derived from the growth of the skull. It has been shown that skull growth in the modern horse may be quantitatively described by a first or second degree equation; furthermore, that the same equation is applicable to skull evolution in the progenitors of the horse. Apparently the changes of skull proportions with growth are identical in ontogeny and phylogeny, hence in the latter case no specific form mutations are believed to have occurred. *Per contra*, in the phylogeny of the foot there is ample evidence for predicating the occurrence of a form mutation producing the single- from the three-toed extremity.

The horse is distinguished from all other animals now living by the fact that he has but one toe on each foot. (We will not here discuss the syndactyl or "mule-foot" mutation of swine, etc., mentioned by Bateson and others.) Comparison with other animals shows that this remaining toe is the third or middle toe of the foot. The hoof corresponds to the nail or claw of other vertebrates and is broadened out to enclose the spatulate distal phalanx technically termed the "coffin-bone". The most proximal of the three phalanges articulates strongly by a ginglymoid joint with the corresponding metacarpal or metatarsal, the so-called "cannon-bone". Behind the cannon-bone and closely adherent to it are two slender spicules, one on each side, the "splint" bones. These are vestiges of the second and fourth digits of the primitive foot, but are so diminished in size that no irregularity of the skin surface indicates their presence beneath.

The splint is composed of four members which consort as a unit, namely, the three phalanges and the corresponding metacarpal or metatarsal (Ewart). In the transition from the form of *Eohippus* with four front- and three hind-toes to *Mesohippus* with only three toes on each

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foot, these four bones were simultaneously involved in unit v. Similarly, in the evolution from the three-toed to the one-toed state all four bones in units ii and iv were symmetrically reduced. The assumption that this group constitutes a genetic unit is further fortified by the known occurrence of a case of incomplete polydactyly (to be reviewed in the next chapter) in which specimen unit ii was well developed, but unit iv remained vestigial and unaffected. If reverse mutations of this sort were verified the evidence indicating this to be a unit character would be complete.

DATA

The fact that any of the smaller foot bones should have been preserved in these fossil Equidae is indeed extraordinary, and it is most surprising that entire sets have been recovered without loss, distortion, or transposition of homologous parts from adjacent skeletons.

The splint of the modern horse and the four bones which it represents in the tridactyl ancestor are contrasted in this study. Its maximum length is readily measured in a prepared skeleton with a technical error of less than 2 per cent. in even the smallest specimens, and approaching $\frac{1}{2}$ per cent. in the largest splints. In the articulated bones of the three-toed specimens there is a systematic error in the measurement of the over-all length of the four bones due to an approximately natural spacing between them, but this is a comparable quantity constant in all and is relatively small. The lengths of the units ii and iv are in every case correlated with the maximum length of the corresponding cannon-bone of the median unit. This length is taken to be, in the mounted specimen, its greatest extent as seen on the lateral aspect. In this case the error of measurement is less than 1 per cent.

One fore- and/or one hind-foot was measured in each of nineteen specimens, including five four-toed, seven three-toed, and four prehistoric and three modern one-toed horses.

The reliability of each observation is independently attested by its internal consistency with measurements of the corresponding splints on other extremities of the same specimen, and by its external consistency with reference to the corresponding parts of other skeletons of a comparable size.

The homogeneity of the data presented in Table I is eloquent testimony to the care and precision exercised in the reconstruction of these fragments by the Department of Vertebrate Palaeontology of the American Museum of Natural History.

DISCUSSION

It has been suggested from time to time that the present monodactyl condition of the equine foot has been progressively approached by minute gradations, thus revealing a directional continuity in its evolution. In this re-examination of the data it has not been possible to consider the fate of digit v because of the paucity of evidence. The structural units ii and iv supply important information, exhibiting two modes of evolutionary change.

TABLE I

Specimen	Museum No.	Fore-foot			Hind-foot		
		"Cannon-bone" meta-carpus iii	"Splint"*		Cannon-bone iii	"Splint"	
			ii	iv		ii	iv
Four-toed species:							
<i>Hyracotherium</i> (<i>Kohippus</i> : Cope)	15431 and 4895 and x }	4.53	6.2	6.2	6.5	9.0	9.0
<i>Orohippus</i>	12648	4.5	6.3	6.0	6.5	—	9.8
<i>Kohippus venticolus</i>	4832	4.9	6.6	6.4	6.8	8.8	9.1
<i>E. resartus</i>	15428	5.6	—	7.7	7.7	—	9.7
<i>Kohippus</i> sp.	15820	—	—	—	7.3	10.3	10.3
Three-toed species:							
<i>Mesohippus bairdi</i>	1492	7.75	10.0	9.9	9.3	—	—
<i>M. intermedius</i>	12454	9.1	—	11.9	12.1	—	—
<i>M. bairdi</i>	12456	9.4	12.2	11.7	10.7	12.9	13.0
	and 1185						
<i>M. intermedius</i>	1196	12.5	16.0	17.0	15.1	18.8	17.5
<i>Merychippus sejunctus</i>	8291	16.3	20.0	19.5	18.1	21.8	21.8
	and 8383						
<i>Hypohippus osborni</i>	9407	20.0	26.2	25.3	(27.4)	27.0	26.7
<i>Neohipparion whitneyi</i>	9815	21.0	25.0	24.7	24.9	28.7	28.7
One-toed horses:							
<i>Pliohippus tull</i>	17225	21.5	*(19.8)	(19.8)	(21.5)	—	19.8
<i>Equus scotti</i>	(panel)	21.0	16.4	16.0	25.0	19.5	19.8
<i>E. scotti</i>	10606	23.0	—	17.1	25.5	—	20.0
<i>Plesippus simplicidens</i>	20076, 7	23.5	—	18.2	26.0	—	17.0
<i>Equus zebra</i> (new-born)	(foal)	18.0	12.3	11.3	20.5	15.1	14.8
<i>E. przewalskii</i>	32686	21.5	15.2	14.5	25.8	15.6	17.8
<i>E. caballus</i> (trotter)	foal 66	23.8	17.0	17.3	27.4	—	20.0
<i>Latopterna</i>							
<i>Diadiaphorus</i> (3 toes)	—	7.6	9.9	9.6	7.4	9.3	9.0
<i>Thoatherium</i> (1 toe)	—	5.8	0.7	0.7	6.5	0.8	0.8

* Unreliable observations bracketed, and not plotted in Fig. 2. Measurements in cm.

In all the polydactyl species here reported the paramedian units ii and iv are definitely shorter than the median unit iii. With increase of total size that discrepancy becomes more and more conspicuous, so that eventually the paramedian digits fail to reach the ground at all. Except in the case of the "three-toed forest horse", *Hypohippus* (which, according to Osborn, lived in marshy regions where the yielding surface brought the other digits into play), these structures, as the individuals

of successive races grew taller, were progressively deprived of function. This condition was the inevitable result of intrinsic differences in the relative growth rates of the parts involved. The situation conforms to an algebraic equation so that given the first few members of the series the final state is predictable. Expressed in terms of an arbitrary standard

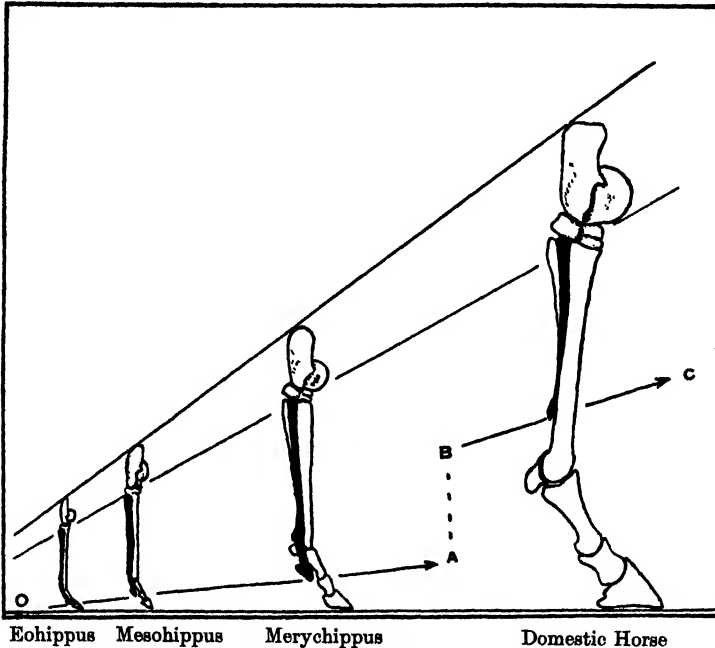


Fig. 1. Two modes of evolution observed in the right hind-foot of the Equidae. Note: progressive elevation of the lateral digit along the mode O-A, thus depriving it of function; an abrupt reduction of the lateral toes to splints (A-B); and the modern ontogeny of the splint directly from O to B to C, omitting the ancestral path O-A.

of reference, i.e. the cannon-bone (CB) of unit iii, the total length of the paramedian units may be given thus:

$$\text{Length of unit ii or iv} = 1.5 (CB)^{0.97 \text{ to } 0.98}.$$

This function applies equally well to either units ii or iv, on either fore- or hind-limbs. Furthermore, it is unaltered in *Hyracotherium* and *Eohippus* despite the presence of digit v on the anterior extremities of these species. In all species the hind-limbs are perhaps 15-20 per cent. longer than the front-limbs (if we can be sure that they belonged to the same individual) but each conforms to the same equation. Given this type of relative growth in the foot we might have predicted the observed

progressive loss of function in the accessory digits as a concomitant to the increased stature characteristic of equine evolution.

In the modern horses, which have been relieved of useless full-sized and encumbering digits, a new equation is required.

$$\text{Splint length} = 0.76 (CB)^{0.99} \text{ or } 1.00.$$

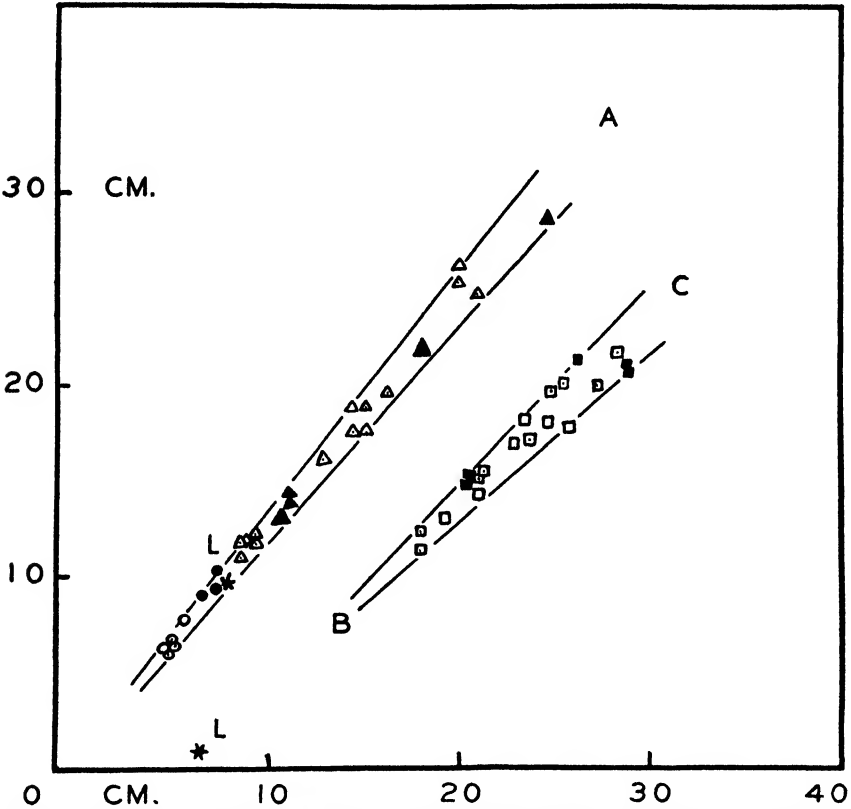


Fig. 2. Length of the paramedial digits or splints, plotted against the length of the cannon bone of the same foot. Overlapping points not shown. Open symbols for front foot; solid symbols for hind foot. Circles, four-toed; triangles, three-toed; squares represent one-toed horses with diminished "splints" growing (from left to right, with reference to cannon-bone increase) at a lower level (B-C) but roughly parallel to those of their ancestors (O-A). Note: *Litopterna* (L*) showing three-toed form quantitatively identical with three-toed horses; one-toed form below, with its splint greatly reduced, suggesting a "parallel mutation".

This description applies to both small and large animals, whether fossil or domestic monodactyls. One is inclined to doubt the significance of that small difference in the magnitudes of the exponents in the two equations. A few more observations might bring up the smaller value

to unity because of the narrow range ($6\times$) of the three-toed series in contrast to the greater range ($200\times$) available for one-toed animals. These data with supplements from embryology will be shown by graph in the next part of these studies.

Reference is commonly made to a "specialization" of the median digit of the horse for weight bearing. This concept is difficult to evaluate. There has been no obvious thickening of the metacarpus or metatarsus with reference to its own length on comparison with the primitive counterpart. The proximal phalanx tends to be long with reference to the cannon-bone in modern horses, but this is also true of some of the primitive animals. Ewart measured the over-all length of the three phalanges of digit iii in ancient and modern species. In each case his data may be expressed by the same equation:

$$\text{Length of three phalanges} = 0.85 (CB)^{0.93}.$$

These negative findings strengthen the impression that the paramedian digits were suppressed without conspicuous effect upon the residual unit iii.

These quantitative observations concerning the two mechanisms of evolution which have operated upon the foot of the horse are illustrated in the accompanying Fig. 1. The divergent lines indicate the role of relative growth trends motivated from left to right, by successive racial size changes. (Presumably these were true size-mutations since one may discount environmental effects which are inadequate to differentiate the sizes of modern Equidae.) But relative growth does not, as in the case of the skull, provide a complete account of form changes in the foot. At that stage of elongation where the accessory digits were deprived of function and became a definite encumbrance upon the foot there is found an abrupt transformation which has the force and effect of a form-mutation. The evidence on this point will be demonstrated by a graph to be presented in the next part.

It may be of interest to note what might be construed as a "parallel mutation" in the *Litopterna*. These are an extinct group of camel-like South American animals. Evolving from a different five-toed ancestor they provide a striking parallel to the development of the horse by gradually increasing in size, lengthening their legs, reducing their outer digits, and from three-toed becoming one-toed. The measurements have but slight value, being made not on the original specimens but from a drawing "one-half natural size" (Matthew). However, these estimates support the view that in the three-toed *Litopterna* the lengths of units

ii and iv bear precisely the same algebraic relation to cannon-bone length which was characteristic of the three-toed horse. The suppression of the paramedian units ii and iv is more drastic in the one-toed *Litopterna* than in the one-toed horse, approximating the condition of unit v as that existed in *Mesohippus*. More search might reveal a series of discontinuous modifications of the primitive pentadactyl foot referable to an allelomorphic series of gene mutations, recurring in diverse evolutionary lines as quantitatively identical parallel mutations. At present the evidence for this supposition is lacking.

SUMMARY

Two modes of evolution are involved in the history of the foot of the horse, continuous and discontinuous.

Continuous variation is in accord with the principles governing the relative growth of parts, whereby any augmentation of total size favours unequally its component parts. Given that digits ii and iv are initially shorter than digit iii in the primitive *Eohippus*, and granted that the apex of the longest digit will support the body weight, it is obvious that progressive elongation of the limb observed in *Mesohippus* and *Merychippus* tends to elevate the paramedian toes and eventually deprives them of an opportunity for weight-bearing.

Discontinuous variation is observed in the abrupt transformation from a three-toed to a one-toed foot. The course of relative growth in the latter case is still algebraic, but a new equation is required.

Whereas the occurrence of "continuous" evolution requires a succession of general size mutations, the "discontinuous" mode of evolution may be attributed to the intervention of a specific form mutation.

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NON-DISJUNCTION IN *OENOTHERA* AND THE GENESIS OF TRISOMICS

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(With Eleven Text-figures)

I. INTRODUCTION

THE present paper embodies the results of a study of the types of non-disjunction and their relative frequency in pollen mother cells of *Oenothera biennis* and a new wild *Oenothera* found by Prof. R. R. Gates at St Valier, Quebec, in 1932. This new type has been given the name *O. laevigata* Bartl. var. *similis* by Prof. Gates (1936) and has a ring of 14 chromosomes at meiosis. *O. biennis* has 2 chromosome rings, one of 6 and one of 8 chromosomes.

The first recorded observations of non-disjunction in *Oenothera* were made by Gates (1908) in pollen mother cells of *O. rubrinervis* and by Davis (1910, 1911) in *O. biennis* and *O. Lamarckiana*. The significance of these observations in relation to the discovery that *O. Lamarckiana* mut. *lata* had 15 somatic chromosomes was soon recognized. By 1923 a considerable number of such trisomics had been recognized, mostly derived from *O. Lamarckiana* (Gates, 1923). For many years the trisomic mutants of *O. Lamarckiana* were classified into 7 groups in agreement with the assumption that there were 7 homologous pairs of chromosomes and that ring formation was a specific genetic property, the differences between the trisomics within any one group being explained as mutations of the germ plasm (de Vries & Boedijn, 1923). Assuming that there were 12 different ring-forming chromosomes, and that each had a specific position in the ring, Hoepfener & Renner (1929) deduced that there should be a possible 13 primary trisomics of *O. Lamarckiana*. After the complex interchange theory of inheritance in *Oenothera* had been propounded (Darlington, 1931), Catcheside (1933) showed that, as a result of double non-disjunction on the same side, a further number of primary trisomics might be expected which would be characterized by breeding true and having a particular maximum catenation at meiosis.

The present work also includes a determination of the maximum possible number of primary trisomics for rings of various sizes based

upon three assumptions: (1) That there are two distinct gametic complexes (Renner, 1917). (2) That each chromosome consists of three parts: two distal pairing segments homologous with the pairing segments of adjacent chromosomes in the ring; and one median differential region, the aggregate of the differential regions accounting for the genetic differences between the two gametic complexes. (3) That the presence of at least one representative of each pairing segment is a condition of gametic viability.

The effect of heterogamy upon the numbers of trisomics possible is discussed, and the origin and properties of possible interchange trisomics are considered. It was hoped to assess the relative frequency of each type of 8-chromosome gamete. Unfortunately the numbers observed are not large enough to be significant.

II. MATERIAL AND METHODS

The plants from which material for cytological examination was collected were grown at Regent's Park, those of *O. laevigata* var. *similis* in 1933, and those of *O. biennis* in 1935. Young flower buds of *O. laevigata* were immersed in Carnoy for 2 or 3 sec., washed in water and fixed in a modified medium Flemming containing uranium trioxide in place of osmic acid. Sepals and petals were stripped off beforehand, and an exhaust pump was used. Preparations were stained by Newton's gentian violet iodine method following bleaching for 24 hours on a hot plate in a mixture of 20 c.c. 20-vol. hydrogen peroxide to 40 c.c. 90 per cent. alcohol.

The preparations of *O. biennis* were smears fixed in Belling's modification of Navashin (Belling, 1930). The smear technique used has been described by Catcheside (1935). This method gave preparations far superior to those obtained by use of the paraffin method.

III. THE TYPES OF NON-DISJUNCTION CYTOLOGICALLY OBSERVABLE IN A RING-FORMING *OENOTHERA*

The normal disjunctional arrangement of the chromosomes at meiotic metaphase in a ring-forming *Oenothera* is well known and is illustrated in Fig. 1. (In the diagrams, pairing segments are unshaded, differential regions shaded.) At anaphase, successive chromosomes in the ring pass to opposite poles, any disturbance of this type of arrangement and separation being non-disjunctional. Many different types of non-disjunction are mechanically possible, but only three have been observed cytologically. Owing to the frequent formation of one or more chromosome

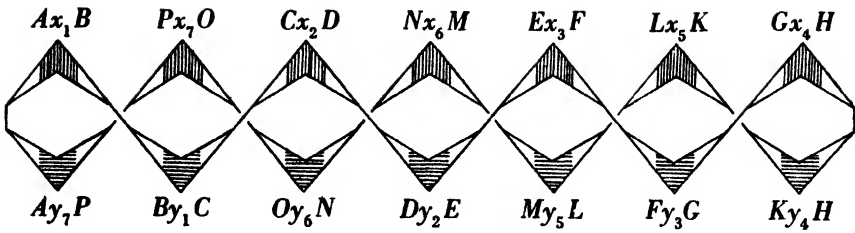


Fig. 1

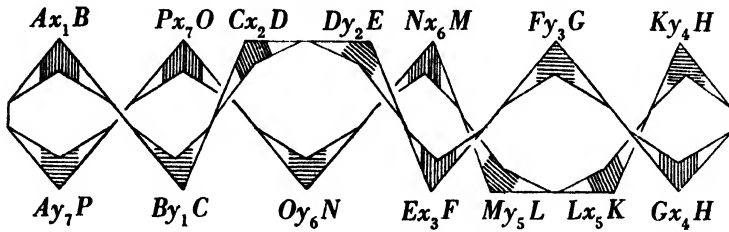


Fig. 2

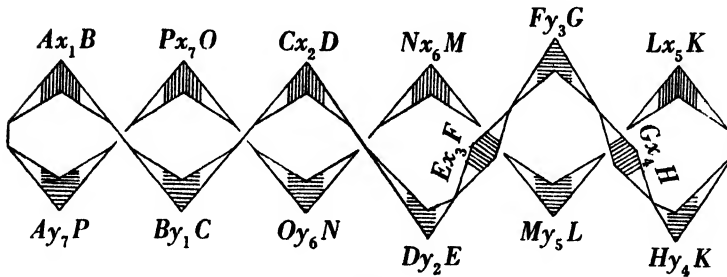


Fig. 3

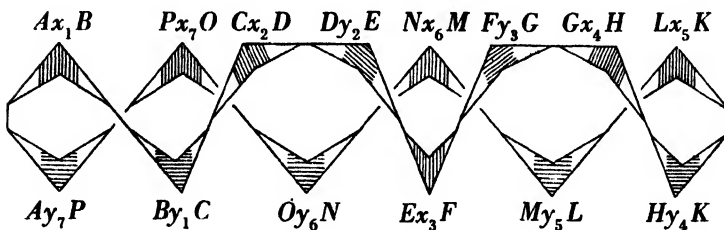
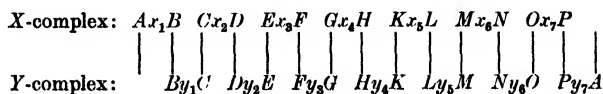


Fig. 4

Figs. 1-4. Disjunctive arrangements of chromosomes in an *Oenothera* with a ring of 14. Fig. 1. Normal. Fig. 2. Double non-disjunction on opposite sides. Fig. 3. Disjunctive arrangement leading to three-in-a-row non-disjunction. Fig. 4. Double non-disjunction on same side, non-disjoining pairs separated by 1 and 9 chromosomes respectively.

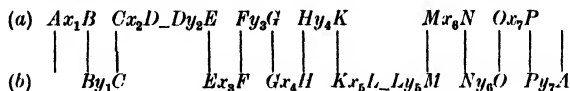
chains in place of the ring, complications of the disjunctional possibilities may occur, though the actual disjunctional arrangements observed in smaller chains are of the same types as those in larger chains and rings. This complication brought about by chain formation will be considered later. For the present we will confine our attention to the three cytologically observable types of non-disjunction.

In order to demonstrate these types of non-disjunction and the differences between the gametes to which they give rise, we will consider an *Oenothera* having a ring of 14 chromosomes and whose gametic complexes, for convenience, will be called *X* and *Y*. The chromosomes may then be written:



Pairing segments are represented by *A*, *B*, etc., and differential segments by x_1 , y_1 , etc., the upper 7 chromosomes constituting the *X*-complex, and the lower 7 the *Y*-complex. Each chromosome is given a differential segment, though, as Catcheside (1936) points out, there is as yet no evidence in support of the hypothesis that *every* chromosome has such a differential segment. The validity of the following deductions is not affected if certain of the chromosomes lack differential regions.

In the first type, double non-disjunction on opposite sides (Fig. 2), two pairs of successive chromosomes pass to opposite poles, thus:

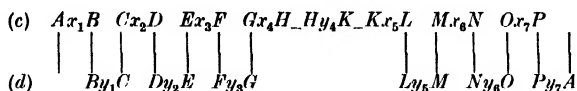


Such a separation leads to the formation of 7-chromosome gametes, each having one or more chromosomes of the one complex replaced by one or more chromosomes from the other complex. In the example, chromosomes Dy_2E , Fy_3G , and Hy_4K of the *Y*-complex have been replaced by chromosomes Ex_3F , Gx_4H , and Kx_5L of the *X*-complex. The number of chromosomes so exchanged obviously depends upon the number of chromosomes in the ring separating the non-disjoining pairs. All gametes formed as a result of this type of non-disjunction lack a pairing segment and are thus inviable. For instance, gamete (a) lacks segment *L*, and gamete (b) lacks segment *D*.

Three varieties of this type are possible in which the non-disjoining pairs of chromosomes are separated by 10 and 0, 8 and 2, and 6 and 4 chromosomes respectively. As, on our assumption, the gametes produced

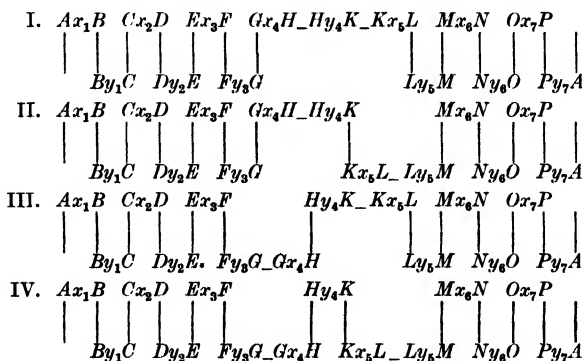
by these varieties of non-disjunction are all inviable, they will not concern us further.

The second type, which may be called "three-in-a-row" non-disjunction, is illustrated in Fig. 3. Three successive chromosomes in the ring pass to one pole, resulting in 8- and 6-chromosome gametes being formed. 8-chromosome gametes of this type will be referred to as type A. They contain all the chromosomes of one complex plus one from the other complex.



In the example, gamete (c) contains the whole complement of X-complex chromosomes plus Hy_4K . It is not deficient in a pairing segment and may be viable. The gamete (d), containing only 6 chromosomes, is inviable. Three-in-a-row non-disjunction can occur at 14 different loci in the ring. Therefore, there are 14 different possible 8-chromosome gametes of this type. Seven of these will be gametes carrying the full X-complex and one Y-chromosome and 7 will be gametes with the Y-complex and one X-chromosome. Thus, in each combination all 14 pairing segments are represented, 2 of them twice; and further, there is present an extra differential segment of the opposite complex.

Reference to Fig. 3 will show that 2 of the 3 non-disjoining chromosomes are suspended between chromosomes which will pass to opposite poles. It frequently happens that the spindle-fibre attachment of these chromosomes gives no indication which way they will move at anaphase. In such cases the suspended chromosomes are regarded as having equal chances of passing to either pole. With the chromosomes arranged as in Fig. 3, there would be even chances of four types of anaphase separation. These are:

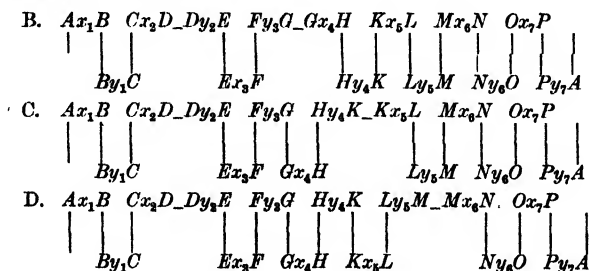


Case I alone gives three-in-a-row non-disjunction. Cases II and III each give double non-disjunction on opposite sides, while case IV gives an example of double non-disjunction on the same side, to be considered shortly.

There is a further possible fate for the suspended chromosomes. When the other chromosomes separate at anaphase, it is quite conceivable that they may occasionally be left on the spindle and behave as univalents. If such univalents divide at either division I or division II (but not at both), the proportions of each type of effective disjunction would not be affected. This is the normal behaviour of univalents, but they may behave in either of two other ways: (1) they may divide at *both* divisions; (2) they may lag at either division and be left out of the tetrad nuclei. Possibility (1) would operate to raise the frequency of 8-chromosome gametes, whilst (2) would tend to lower it. These two possibilities are remote, the first probably more so than the second, and as there are no available quantitative data upon the frequency and behaviour of univalents in *Oenothera*, they will be disregarded.

The third type of non-disjunction, styled double non-disjunction on the same side, is characterized by two pairs of successive chromosomes passing to the same pole and also results in an 8-6 numerical separation at anaphase. The non-disjoining pairs may be separated by 1 and 9, 3 and 7, or 5 and 5 chromosomes (Figs. 4, 5 and 6). In the first two cases there are 14 possible positions in the ring and thus a possible 14 different 8-chromosome gametes from each. Where the non-disjoining pairs are separated by 5 chromosomes on both sides, there are only a possible 7 different positions in the ring (the eighth position repeats the first). In this case only 7 different 8-chromosome gametes can be formed.

The 8-chromosome gametes arising as a result of these three variations of double non-disjunction on the same side will be referred to as types B, C, and D. Examples are:



It will be seen that in case B, one X-complex chromosome has been

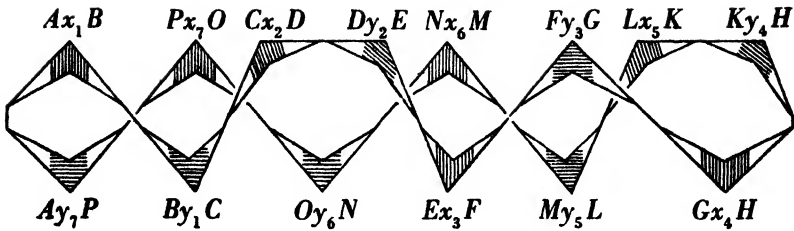


Fig. 5

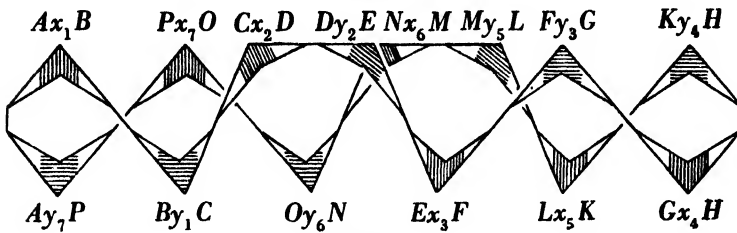


Fig. 6

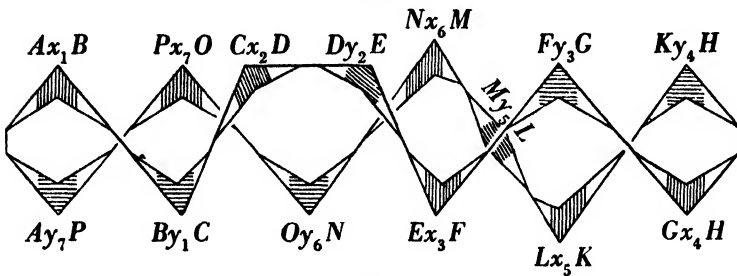


Fig. 7

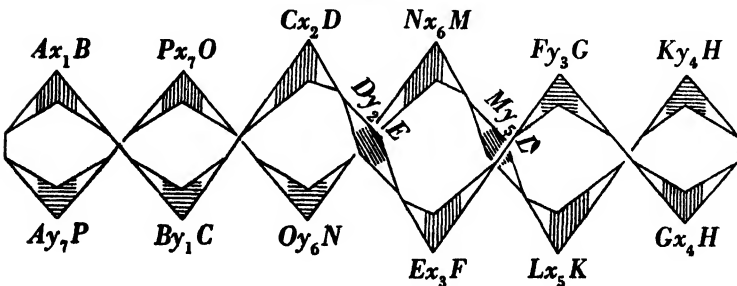


Fig. 8

Figs. 5-8. Disjunctional arrangements of the chromosomes in an *Oenothera* with a ring of 14. Fig. 5. Double non-disjunction same side, non-disjoining pairs separated by 3 and 7 chromosomes respectively. Fig. 6. Same, non-disjoining pairs separated by 5 and 5 chromosomes respectively. Fig. 7. Disjunctional arrangement involving one non-disjoining pair and a suspended chromosome. Fig. 8. Disjunctional arrangement involving two suspended chromosomes separated by more than one chromosome (cf. Fig. 3).

replaced by two adjoining *Y*-complex chromosomes; in case C, two *X*-chromosomes have been replaced by three *Y*; and in case D, three *X*-chromosomes by four *Y*. In all of these gametes, twelve of the pairing segments occur once, and two of them twice. In this respect they resemble the 8-chromosome gametes formed as a result of "three-in-a-row" non-disjunction. But, in addition, and as a result of exchange of chromosomes between opposite complexes, 1, 2 or 3 differential segments of the one complex are exchanged for 2, 3 or 4 differentials of the other complex.

The further abnormal 8-6 chromosome distributions at anaphase possible as a result of combinations of the above disjunctional types, with or without chain formation, can only give 8-chromosome gametes lacking at least one pairing segment. These are inviable. Thus only effective disjunctions of types A, B, C and D give rise to viable 8-chromosome gametes. As there are no marked size differences or other morphological peculiarities distinguishing the chromosomes of the ring, the determination of the precise constitution of the various 8-chromosome gametes is impossible. Only the gametic *type* is determinable.

Summing up, as a result of four varieties of non-disjunctional separation at anaphase, four different types of 8-chromosome gametes can arise. 14 different gametes of each of types A, B and C, and 7 of type D are possible, making a total of 49. These gametes agree in that all the pairing segments are represented, two of them occurring twice. They differ from one another in respect of the differential segments they contain and the pairing segments for which they are duplex. In each of the types A, B and C, 7 gametes may be regarded as modified *X*, and 7 as modified *Y*-gametes. Type D gametes cannot be so regarded, as they each contain 4 chromosomes of each complex.

It is advisable to test the number of viable 8-chromosome gametic types determined in this way by solving the problem, "In how many ways may eight pairs of letters be chosen from: *AB, BC, CD, DE, EF, FG, GH, HK, KL, LM, MN, NO, OP, PA*, so that each letter is included at least once?"

In order to satisfy the condition that every letter occurs once, two letters must occur twice and only twice in each case.

When *A* is duplicated, *C, E, G, K, M, O* cannot be duplicated, for such duplication involves a third duplication, and therefore a deficiency. Therefore *A* can be duplicated with *B, D, F, H, L, N, P*, making seven combinations.

Similarly, there are seven combinations in which each of *B, C, D, E, ... P*, can be duplicated, making a total of 14×7 combinations.

But two letters are duplicated in each case, so each combination will occur twice.

Therefore, the total number of combinations is $\frac{14 \times 7}{2} = 49$.

IV. OBSERVATIONS ON *OENOTHERA LAEVIGATA* VAR. *SIMILIS*

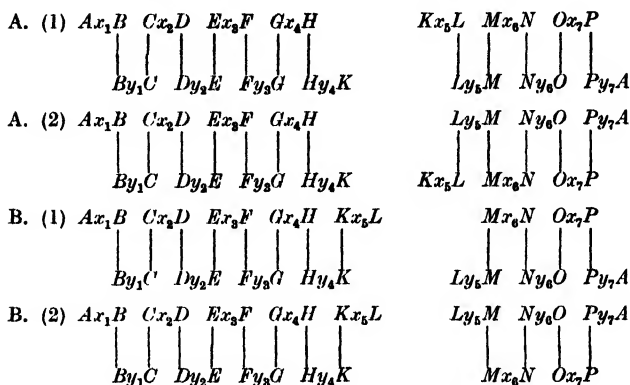
The results of an examination of 65 full metaphase or early anaphase nuclei are summarized in Table I. The actual disjunctional arrangements observed are rarely as clear as the diagrams indicate, and so, before giving an account of these results, an explanation will be given as to how the effective types of disjunction were determined.

Considering an individual chromosome, there are two positions, other than the normal, in which it may occur at metaphase. In the one position, the drawn-out appearance of its spindle-fibre attachment definitely indicates that it will pass to the same pole as one adjacent chromosome in the ring, thus giving a non-disjoining pair. In the other position, it is suspended between chromosomes destined to pass to opposite poles, and its spindle-fibre attachment gives no indication as to which way it will move at anaphase. Such a chromosome may pass to either pole at random, or behave as a univalent and divide at either division I or division II (see above, three-in-a-row non-disjunction). Each of these three possibilities gives equal chances of the suspended chromosome being included in one group of disjoining chromosomes or in the other. "Suspended" chromosomes then, for the purpose of analysing the effective disjunctional arrangements, may be regarded as passing at random to one pole or the other at anaphase.

The effective types of non-disjunctional arrangement, considered in the previous section, all arise from combinations of these two abnormal positions of a chromosome in the ring. Thus, two non-disjoining pairs may give either double non-disjunction on the same side (Figs. 4, 5 and 6) or on opposite sides (Fig. 2); three-in-a-row non-disjunction can occur when two suspended chromosomes are separated by one orientated chromosome (see above) (Fig. 3); while one non-disjoining pair and a suspended chromosome (Fig. 7), or two suspended chromosomes separated by more than one orientated chromosome (Fig. 8), give equal chances of double non-disjunction on the same side and on opposite sides.

In a complete ring, or a chain of 14, determination of the disjunctional possibilities is straightforward. Where two or more chains are formed in place of the ring, a further complexity occurs. As examples, we will examine the cases of (A), chains of 8 and 6 chromosomes, and

(B), chains of 9 and 5 chromosomes, all disjunctionally arranged. In each case there are two disjunctional possibilities, namely:



A (1) and B (1) are normal. A (2) is a case of double non-disjunction on opposite sides which cannot be detected cytologically. B (2) is a case of double non-disjunction on the same side giving an 8-6 numerical disjunction at anaphase, and is, therefore, detectable. If the two chains in each case are orientated independently, then equal proportions of each disjunctional possibility may be expected. This will be assumed where the ring is replaced by two even-numbered chains. Where odd-numbered chains occur, it is capable of being tested. The actual numbers observed were 9 disjoining normally, to 2 disjoining abnormally to give non-disjunction on opposite sides. These figures do not support the hypothesis of independent orientation of the chains, but they are scarcely large enough to be significant.

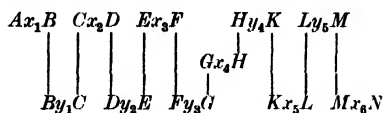
As an example of the application of the assumptions considered above to a concrete case, nucleus number 52 (Fig. 9) will be considered in detail.



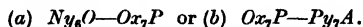
Fig. 9. Nucleus no. 52. Chain of 11 with one suspended chromosome, one chain pair, and a univalent, $(11) + (2) + 1$. The univalent is drawn separately.

In this nucleus the ring was broken in three places giving chains of 11 and 2, and a univalent. Further, there was one "suspended" chromo-

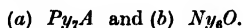
some in the chain of 11 which had not established a connexion with either pole. If we regard the chain of 11 as:



then the chain of 2 must be either:



With both chains fixed, there is only one position in the ring for the univalent in each case, namely:



The disjunctional possibilities are: (1) the suspended chromosome in the chain of eleven may pass to either pole at anaphase; (2) the chain of two may be derived from either of two positions in the ring; (3) this chain may disjoin in either of two ways compared with the chain of 11; (4) the univalent may pass to either pole at anaphase. Thus, in this nucleus there are 16 different disjunctional possibilities, each giving rise to two different gametes. These gametes are of five effective types, viable 8-chromosome gametes of types C and D and deficient inviable gametes containing 6, 7 or 8 chromosomes.

Each nucleus examined has been subjected to such an analysis, the results of which are presented in Table I. The possible gametic combinations to which each nucleus may give rise have been tabulated under the effective disjunctional arrangements which are a condition of their origin.

Table I shows that the frequency of non-disjunctional arrangements is considerably lower in complete rings than in cases where the ring has been replaced by one or more chains. This is partially due to random arrangement of two or three chains, but is not due to such a factor alone which cannot operate where one chain only is concerned. Here, the increase in non-disjunction frequency as compared with the ring is accounted for by an increase in frequency of abnormal chromosome arrangements within the association.

The frequencies of the various types of gametes produced are also shown in Table I. The figures for the 8-chromosome gametes indicate that types A and B occur approximately twice as frequently as types C and D, but probably a much larger number of observations is necessary in order to establish a significant difference in the ratio of one such type to another.

The total percentage of normal disjunctions is 54 per cent., which represents the minimum percentage of good pollen in the absence of a pollen lethal factor. As the 8-chromosome combinations produced in pollen meiosis may conceivably become good pollen grains, though failing to function in pollination, the percentage of such gametes should be included in the maximum percentage of good pollen. The percentage of 8-chromosome gametes is 12. Therefore the maximum percentage of good

TABLE I

Disjunctional arrangements in Oenothera laevigata var. similis (ring of 14)

		Disjunctional arrangements giving							
Catenation	No. nuclei examined	Normal gametes	In viable 7-chromosome gametes	8-chromosome gametes, type A	8-chromosome gametes, type B	8-chromosome gametes, type C	8-chromosome gametes, type D	In viable 8-chromosome gametes	9-chromosome gametes
(13)	11	8	—	—	—	—	—	—	—
(14)	30	15	6½	3½	2¾	4	1½	—	—
(13) + 1	1	—	—	—	½	—	—	—	—
(12) + (2)	1	½	½	—	—	—	—	—	—
(11) + (3)	4	4	—	—	—	—	—	—	—
(10) + (4)	4	1	2	½	—	½	—	—	—
(9) + (5)	5	4	1	—	—	—	—	—	—
(8) + (6)	4	1	1	1	—	½	½	—	—
(7) + (7)	2	1	1	—	—	—	—	—	—
Total (2 breaks)	21	11½	5½	1½	½	1	½	—	½
(11) + (2) + 1	1	—	½	—	—	¼	¼	¼	—
(7) + (4) + (3)	1	½	½	—	—	—	—	—	—
(8) + (5) + 1	1	—	—	—	¼	¼	—	—	½
Total (3 breaks)	3	½	1	—	¼	¼	—	¼	½
Total	65	35	13	4¾	5½	2¼	2½	¾	1
Gametic frequencies		140	52	9½	11	4¾	5¼	1¼	2
Gametic percentages		54	20	12				1	1

pollen grains is 66. The observed value from a count of approximately 300 grains was 60 per cent., which is, therefore, in very good agreement with the figures determined from non-disjunction.

Out of the sixty-five nuclei examined there were two which could disjoin at anaphase into groups of 9 and 5 chromosomes. Neither of the possible 9-chromosome gametes would have been deficient in a pairing segment, and therefore their chances of becoming viable megaspores would depend upon the balance of the differential and pairing segments present. 9-chromosome megaspores may be viable in triploids, as evi-

denced by chromosome counts on the progeny of triploid-diploid crosses (Dulfer, 1926; Capinpin, 1933).

V. NON-DISJUNCTION IN *OENOTHERA BIENNIS*

Observation on rings of 6 and 8 chromosomes is much simpler. In each case there are only two non-disjunctional types which can give rise to viable 8-chromosome gametes. One of these is three-in-a-row non-disjunction, the other, double non-disjunction on the same side. In the latter type, in the ring of 6, the non-disjoining pairs are separated by one chromosome on each side; while in the ring of 8 they are separated by one and three chromosomes respectively. The observations are summarized in Tables II and III.

TABLE II

Non-disjunction in the ring of 6. (Oenothera biennis)

Catenation	No. of nuclei examined	Normal	Double non-disjunction on opposite sides	Double non-disjunction on the same side	Three-in-a-row non-disjunction	Total abnormal disjunctions
(6)	52	52	—	—	—	—
(6)	18	16	2	—	—	2
(3) + (3)	2	2	—	—	—	—
Total	72	70	2	—	—	2

TABLE III

Non-disjunction in the ring of 8. (Oenothera biennis)

Catenation	No. of nuclei examined	Normal	Double non-disjunction on opposite sides	Double non-disjunction on the same side	Three-in-a-row non-disjunction	Total abnormal disjunctions
(8)	42	41	—	1	—	1
(8)	22	17½	2	1½	1	4½
(7) + (1)	2	½	½	½	½	1½
(6) + (2)	3	1½	1½	—	—	1½
(5) + (3)	3	1	—	2	—	2
Total	72	61½	4	5	1½	10½

It will be seen that the frequency of non-disjunction in the ring of 6 chromosomes is appreciably lower than in the ring of 8. The combined frequency is 17 per cent. which, again, is considerably lower than in the ring of 14 (46 per cent.).

From this the tentative conclusion may be drawn that the frequency of abnormal disjunctions increases with increasing number of chromosomes in the ring.

VI. THE PRIMARY TRISOMICS DERIVABLE FROM AN *OENOTHERA*
WITH A RING OF 14 CHROMOSOMES

The possible types of 8-chromosome gametes which may be formed by a plant having a ring of 14 chromosomes have been indicated above. In the first place there are the 8-chromosome gametes of type A, arising as a result of "three-in-a-row" non-disjunction. These, it will be remembered, each have all the chromosomes of one complex plus one chromosome from the other complex, and may be of 14 different kinds. When mated with a normal gamete of the opposite complex they will give rise to zygotes having 13 chromosomes represented once and one chromosome represented twice; e.g.



Evidently, 14 different trisomics of this type are possible, one corresponding to each different gamete of type A, or, stated differently, one corresponding to each chromosome of the ring. They should have the maximum catenation illustrated in Fig. 10 a. Cytological studies on trisomics of this type have been made by Catcheside (1933) and Gates & Nandi (1935). The maximum catenation has not been observed in a single case, but may be inferred from the smaller associations seen.

As such trisomics contain all the chromosomes of the parent diploid organism, they may be expected to produce functional gametes of both complexes, in addition to 8-chromosome gametes, and thus be capable of segregating the parent diploid. De Vries (1916) gave the class name "dimorphic" to trisomics of *O. Lamarckiana* which behaved in this way when selfed. In respect of this capacity for segregating the parent diploid they resemble the primary trisomics of *Datura stramonium* (Blakeslee, 1930) and of other structurally homozygous plants.

Catcheside (1936) has adduced evidence to show that some, at least, of the 15-chromosome zygotes formed as a result of mating a *Lamarckiana* gamete of the type:

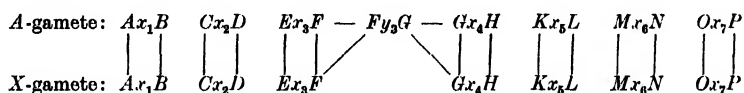
$Av_1B \quad Cv_2D \quad Ev_3F \quad Fg_3G \quad Gv_4H \quad Kv_5L \quad Mv_6N \quad OP$

with a normal *velans* gamete are viable. The condition of viability is, presumably, that the *gaudens* differential segment contained within the extra *gaudens* chromosome (in the example, Fg_3G) should add sufficient *gaudens* genes to convert the lethal balance of the homozygous *velans* zygote into a vital trisomic balance.

In a consideration of the breeding behaviour of this trisomic type,

he has shown that as a class they should breed true. Further, they should be characterized by the chromosome configuration shown in Fig. 10 *b* at meiosis. He has suggested the name "monomorphic" for all the true-breeding trisomics. This type he calls "Monomorphic-I".

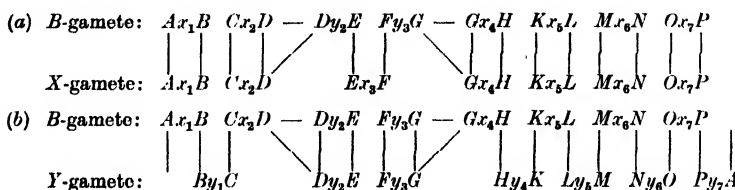
It follows that certain of the 15-chromosome zygotes of this type derivable from a plant with a ring of 14, may be viable. We know that 14 different gametes of type A may be formed, 7 with a complete X-complex, plus one Y-chromosome, and 7 with a complete Y-complex plus one X-chromosome. These gametes, when mated with normal Y- and normal X-gametes respectively will give rise to 14 different 15-chromosome zygotes, which, if viable, will become monomorphic-I trisomics. Example:



The maximum catenation possible in this trisomic is 5 ring pairs plus an association of 5, which is the same as that predicted by Catcheside for the monomorphic-I trisomics of *O. Lamareckiana* (Fig. 10 *b*).

The 8-chromosome gametes of types B, C and D which arise as a result of double non-disjunction on the same side may also be mated with both X- and Y-gametes. The 35 different gametes of these three types will give 70 different zygotic combinations of six different kinds when so mated. Examples of each kind are given below.

It has already been pointed out that 7 of the 14 type B gametes are modified X-, and 7 modified Y-gametes. These when mated with normal Y- and X-gametes respectively will give rise to zygotes of types differing in their degree of heterozygosity. Examples are:



The maximum catenations possible in these zygotic combinations are shown in Figs. 10 *c* and 10 *g*. Combination (a) gives 4 ring pairs and an association of 7. Combination (b) gives an association of 15. Trisomics of these two types will be called monomorphic-II and monomorphic-VI trisomics respectively. Fourteen of each type are possible.

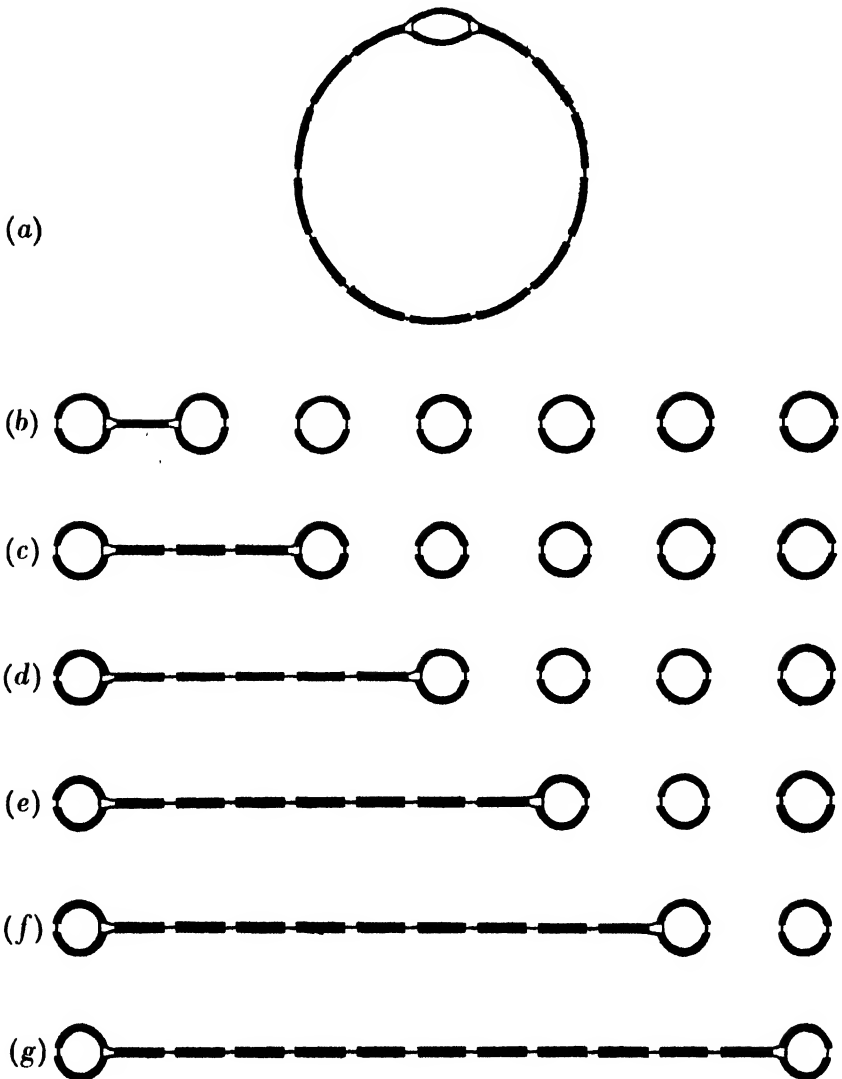
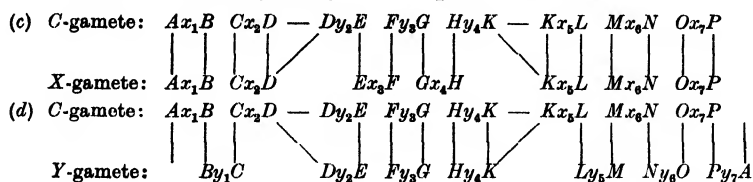


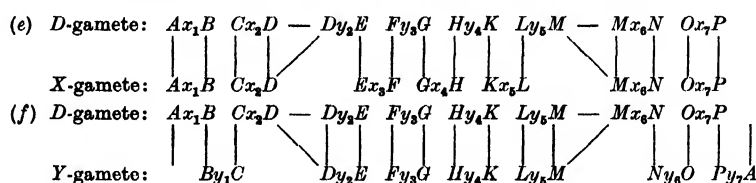
Fig. 10. Expected maximum catenations of primary trisomics derived from an *Oenothera* with a ring of 14. (a) Dimorphic. (b) Monomorphic-I. (c) Monomorphic-II. (d) Monomorphic-III. (e) Monomorphic-IV. (f) Monomorphic-V. (g) Monomorphic-VI.

The gametes of type C contain 5 chromosomes from one complex and 3 from the other. When mated with normal gametes of both complexes they also will give two series of 15-chromosome zygotes which will differ in their degree of heterozygosity. Examples of these are:



Trisomics like (c) should have 3 ring pairs and an association of 9 at meiosis (Fig. 10 d), whilst trisomics like (d) should have 1 ring pair and an association of 13 (Fig. 10 f). Trisomics of these two types will be called monomorphic-III and monomorphic-V trisomics respectively. There will be a maximum possible 14 of each type, corresponding to the 14 different gametes of type C.

The final type of 8-chromosome gamete which is viable on our assumption is type D. Such gametes contain four chromosomes from each complex, and when mated with normal X- and Y-gametes respectively, will give but one type of 15-chromosome zygote. In this respect they differ from the gametes of types A, B and C. For example:



Both (e) and (f) have the same maximum catenation, namely, 2 ring pairs and an association of 11 (Fig. 10 e). Trisomics of this cytological type will be called monomorphic-IV trisomics. There are 7 different gametes of type D, which, mated with X- and Y-gametes respectively, give a possible 14 trisomic combinations of this type.

Summing up, gametes of types A, B and C contain 7, 6 and 5 chromosomes respectively of one complex, plus 1, 2 and 3 chromosomes respectively of the other complex. When mated with normal X- and Y-gametes they should each give rise to two series of 15-chromosome zygotes, 14 different combinations being possible in each series. These series differ in their degree of heterozygosity, which is expressed cytologically in the number of ring pairs possible at meiosis. Gametes of type D, on the other hand, contain 4 chromosomes from each complex and thus should

give rise to only one series of 15-chromosome zygotes when mated with normal *X*- or normal *Y*-gametes. Fourteen trisomics of this series are possible. They also should have a characteristic chromosome catenation at meiosis.

Thus a total of 98 possible trisomics are derivable from a species having a ring of 14. Their origin is summarized in Table IV.

TABLE IV

The types of trisomics derivable from an Oenothera with a ring of 14

Effective disjunctional arrangement	Chromosome constitution of derived 8-chromosome gametes	Trisomic type produced when mated with	
		<i>X</i> -gamete	<i>Y</i> -gamete
Three-in-a-row	<i>X</i> -complex + 1 <i>Y</i> -chromosome <i>Y</i> -complex + 1 <i>X</i> -chromosome	Monomorphic-I Dimorphic	Dimorphic Monomorphic-I
Double non-dis- junction on same side A	6 <i>X</i> - + 2 <i>Y</i> -chromosomes 6 <i>Y</i> - + 2 <i>X</i> -chromosomes	Monomorphic-II Monomorphic-VI	Monomorphic-VI Monomorphic-II
Double non-dis- junction on same side B	5 <i>X</i> - + 3 <i>Y</i> -chromosomes 5 <i>Y</i> - + 3 <i>X</i> -chromosomes	Monomorphic-III Monomorphic-V	Monomorphic-III Monomorphic-V
Double non-dis- junction on same side, type C	4 <i>X</i> - + 4 <i>Y</i> -chromosomes	Monomorphic-IV	Monomorphic-IV

One of the seven series of trisomics is dimorphic, that is, capable of producing functional gametes of both complexes, and so, capable of segregating the parent diploid when selfed. The remaining six series are monomorphic. They should form two kinds of gametes, normal gametes of one complex and 8-chromosome gametes of the kind which contributed to their parentage. As only normal gametes are functional on the pollen side, they should breed true (see Catcheside, 1936). Further, they should be characterized by the production of at least 50 per cent. empty seeds, corresponding to the homozygous zygotes formed as a result of random gametic mating.

Of the 84 possible monomorphic trisomics, half should be capable of producing functional gametes of the one complex, and half gametes of the other complex.

It has already been stated that the trisomics of each series should give characteristic maximum chromosome catenations at meiosis. These are shown diagrammatically in Fig. 10. The dimorphic trisomics have one ring-forming chromosome duplicated and so may be expected to give a ring pair joined to the ring by two triple unions as the maximum configuration possible.

The monomorphic trisomics should show as a maximum catenation

a variable number of free ring pairs plus an association in which 2 ring pairs are joined by a chain composed of the odd numbers from 1 to 13 chromosomes, the number of free ring pairs and the number of chromosomes in the chain being a characteristic of each series. This general type of maximum association in monomorphic trisomics was predicted by Catcheside (1933). Owing to breaks in the maximum expected configuration in each of these series, smaller associations should be observed from which the maximum possible association may be inferred. Thus each series should have a particular maximum number of free ring pairs and a fixed number of chromosomes in the longest possible derived chain. Also, variation in respect of the maximum number of independent associations containing triple chiasmata is possible. The expected derived configurations from each series of trisomics are given in Table V.

TABLE V

The expected derived configurations from each series of trisomics of an Oenothera with a ring of 14

Trisomic series	Maximum number of free ring pairs	Maximum number of chromosomes in longest derived chain	Maximum number of independent associations containing triple chiasmata
Dimorphic	1	15	1
Monomorphic-I	7	5	1
Monomorphic-II	6	7	2
Monomorphic-III	5	9	2
Monomorphic-IV	4	11	2
Monomorphic-V	3	13	2
Monomorphic-VI	2	15	2

As yet, no true-breeding trisomic derived from a plant with a ring of 14 has been examined cytologically, though a true-breeding trisomic of *O. Lamarckiana* (mut. *curta*) was studied by Håkansson (1930). The observations made, so far as they go, are compatible with the predicted maximum catenation of a monomorphic-V trisomic (see Catcheside, 1936).

VII. THE PRIMARY TRISOMICS DERIVABLE FROM OENOTHERAS WITH VARIOUS SIZED RINGS

Species and mutants of the genus *Oenothera* sect. *Onagra* are now known with every degree of structural complexity, ranging from 7 pairs to a ring of 14. The maximum possible number of primary trisomics derivable from each type of ring is given by the expression $n^2/2$, where n is equal to the number of chromosomes in the ring. The number of

these trisomics and the cytological series to which they belong are shown in Table VI.

Reference to this table shows that for each size of ring there are a number of dimorphic trisomics possible equal to the number of chromosomes involved in the ring. Every dimorphic trisomic contains all the chromosomes of the ring, one being duplicated, and is derived from a gamete formed as a result of three-in-a-row non-disjunction.

The monomorphic trisomics are derived largely from gametes formed as a result of double non-disjunction on the same side, but partly (mono-

TABLE VI

The number of possible primary trisomics involving extra ring-forming chromosomes of Oenotheras having rings of different sizes

No. of chromosomes in the ring	Number of 8-chromosome gametes possible from each type of non-disjunction				Number of trisomics possible						
	Double non-disjunction on same side										
	Three-in-a-row non-disjunction	Non-disjoining pairs separated by one chromosome	Non-disjoining pairs separated by three chromosomes	Non-disjoining pairs separated by five chromosomes	Dimorphic	Monomorphic-I	Monomorphic-II	Monomorphic-III	Monomorphic-IV	Monomorphic-V	Monomorphic-VI
2	1 type possible	—	—	—	1	—	—	—	—	—	—
4	4	—	—	—	4	4	—	—	—	—	—
6	6	3	—	—	6	6	6	—	—	—	—
8	8	8	—	—	8	8	8	8	—	—	—
10	10	10	5	—	10	10	10	10	10	—	—
12	12	12	12	—	12	12	12	12	12	12	—
14	14	14	14	7	14	14	14	14	14	14	14
											Total
											98

morphics of class I) from gametes arising from three-in-a-row non-disjunction. The number of different possible 8-chromosome gametes formed in this way, and thus the number of monomorphic trisomics possible, increases with an increasing number of chromosomes involved in the ring. These monomorphics, notwithstanding the size of the ring from which they were derived, should be of the same cytological types as those derived from the ring of 14; for they vary in their chromosome constitution in the same way as the monomorphics derived from the plant with a ring of 14 considered above.

On the other hand, the dimorphic trisomics derived from different sized rings should have different maximum catenations. These should

be similar in general type to that characteristic of the dimorphics derived from the ring of 14 forming plant, but should differ in the number of chromosomes involved in the diagnostic associations. For instance, the dimorphics derivable from the ring of 4 should have a ring pair joined to a ring of 4, while those derivable from the ring of 6 should show a ring pair joined to a ring of 6 as the maximum catenation. Similar maximum associations involving 11 and 13 chromosomes should be found in the dimorphics derivable from plants with rings of 10 and 12 respectively.

The cytology of several dimorphic trisomics of *O. Lamarckiana* has been studied by Håkansson (1930). His observations are compatible with the maximum catenation predicted for dimorphics of a plant having a ring of 12 chromosomes.

VIII. THE PRIMARY TRISOMICS OF HETEROGAMOUS OENOTHERAS

It is well known that some species of *Oenothera* (e.g. *O. muricata*) have functional pollen of one complex only; and functional megaspores only of the other complex. Such species were called by de Vries heterogamous. The determinations of numbers of primary trisomics possible for rings of various sizes given above only applies to isogamous *Oenotheras*. The maximum number to be expected from heterogamous species will be lower in each case. *O. muricata* (*rigens*, *curvans*), which has a ring of 14, will serve as an example. Only the *rigens* complex is functional on the female side and only *curvans* on the male side.

As a result of non-disjunction, 8-chromosome gametes varying in constitution from 7 *curvans* chromosomes plus 1 *rigens* chromosome, to 7 *rigens* plus 1 *curvans* chromosome, may be formed on both pollen and megaspore sides. Such gametic combinations are not functional as pollen but are not necessarily lethal as megaspores, their chances of becoming functional megaspores depending upon the genetic conditions determining the lethality of the *curvans* megaspores.

There are two possible hypotheses by which the lethality of the *curvans* megaspores may be explained. On the one hand it may be caused by a gametic lethal gene; or, alternatively, it may be due to the lethal balance of the aggregate of *curvans* differential segments in a particular environment, the ovule.

The second hypothesis does not exclude the possibility of any particular 8-chromosome combination being viable, for the *rigens* chromosome or chromosomes may introduce sufficient *rigens* genes to restore a vital balance in each case (compare monomorphic-I trisomics, above).

Hence we may regard all the 8-chromosome megaspores not deficient in a pairing segment as *possibly* viable. It was shown above that 49 such combinations are possible in a plant with a ring of 14. These may only be mated with *curvans* pollen, thus giving 49 possible 15-chromosome zygotes, which, if viable, would become trisomics of seven different series, one dimorphic and six monomorphic, with seven forms in each. The possibilities are shown in Table VII. As these trisomics are of analogous chromosome constitution to those of the isogamous plant with a ring of 14, the maximum catenation will be the same in each series.

TABLE VII

Numbers of possible Oenothera muricata trisomics on the assumptions that megaspore lethality is determined by: (a) lethal balance of the aggregate of curvans differential segments; (b) absence of a single vital rigens factor.

8-chromosome gametic combinations produced on megaspore side			Trisomics formed when mated with <i>curvans</i> pollen		
Chromosome constitution	No. possible		Type	No. possible	
	(a)	(b)		(a)	(b)
7 <i>rigens</i> + 1 <i>curvans</i>	7	7	Dimorphic	7	7
6 <i>rigens</i> + 2 <i>curvans</i>	7	6	Monomorphic-VI	7	6
5 <i>rigens</i> + 3 <i>curvans</i>	7	5	Monomorphic-V	7	5
4 <i>rigens</i> + 4 <i>curvans</i>	7	4	Monomorphic-IV	7	4
3 <i>rigens</i> + 5 <i>curvans</i>	7	3	Monomorphic-III	7	3
2 <i>rigens</i> + 6 <i>curvans</i>	7	2	Monomorphic-II	7	2
1 <i>rigens</i> + 7 <i>curvans</i>	7	1	Monomorphic-I	7	1

If the first hypothesis be true, then one *rigens* chromosome must be supposed to contain the vital allelomorph¹ of the lethal *curvans* gene, in which case only one of the 7 *curvans* plus 1 *rigens* combinations, two of the 6 *curvans* plus 2 *rigens*, three of the 5 *curvans* plus 3 *rigens*, and so on, can be viable, for only the number stated for each type will contain the vital *rigens* gene. These gametic combinations when mated with *curvans* pollen will give as a maximum possible, 1 monomorphic-I, 2 monomorphic-II, 3 monomorphic-III, and so on up to 7 dimorphic trisomics, as shown in Table VII.

If the presence of two or more specific chromosomes is a necessary condition of viability of the *rigens* megaspores, then the number of possibly viable 8-chromosome combinations will be reduced still further, but will

¹ The term "allelomorph" is used in a rather extended sense here, as the vital and lethal genes do not cross-over, for which reason they are assumed to occupy non-pairing loci in differential segments.

be predictable from the number of *rigens* chromosomes involved in the balanced lethal system.

The predicted breeding behaviour of monomorphic and dimorphic trisomics respectively of *O. muricata* is shown in Table VIII. The dimorphic when selfed or pollinated by *muricata* should give approximately equal numbers of *muricata* and the parent trisomic. It will be noticed that no monomorphic-I plants are to be expected among the progeny, in contrast to expectation from isogamous dimorphic trisomics. This is due to the fact that the extra chromosome in each dimorphic is a *curvans* chromosome, and such a dimorphic when pollinated with *curvans* pollen cannot segregate a monomorphic-I trisomic.

TABLE VIII

Expected breeding behaviour of Oenothera muricata trisomics when selfed, or pollinated by O. muricata. Dimorphic: constitution curvans rigens + 1 curvans chromosome (written c). Monomorphic: constitution curvans + 8-chromosome complex.

Trisomic	Megaspores formed		Offspring when selfed or pollinated by <i>O. muricata</i> (<i>curvans</i> pollen)
	Type	Viability	
Dimorphic	<i>curvans</i>	Invisible	—
	<i>rigens</i>	Viable	<i>rigens.curvans</i> (<i>O. muricata</i>)
	<i>curvans</i> + c	Invisible	—
	<i>rigens</i> + c	Viable	<i>rigens.curvans</i> + c (Dimorphic)
Monomorphic	<i>curvans</i>	Invisible	—
	8-chromosome complex	Viable	<i>curvans</i> . 8-chromosome complex (Monomorphic)

A *muricata* monomorphic must be derived from a *curvans* complex on the male side and an 8-chromosome gametic combination on the female side. Such a trisomic is normally capable of forming only *curvans* gametes, and similar 8-chromosomes combinations. As the former are non-functional on the megaspore side, and the latter non-functional on the pollen side, it should breed true. Other trisomic forms are to be expected occasionally from both monomorphic and dimorphic trisomics when selfed, owing to non-disjunction in the chromosome chains or rings involved. It will be evident that the types of trisomics to be expected in this way are also predictable for each cytological series of trisomics.

Oenothera species are known which are incompletely heterogamous. For example, in *O. eriensis* (*glaucens.undulans*) and *O. novae-scotiae* (*grandiflorens.parviflorens*), both complexes may function on the megaspore side, but only one on the pollen side (Gates & Catcheside, 1932). If both complexes could function as pollen, the maximum number of

primary trisomics possible would be the same as for an isogamous species with a ring of the same size. In species where only one type of pollen functions, but both types of egg, there are the same possibilities as in a completely heterogamous plant (on the hypothesis of balance of differential segments being responsible for gametic elimination). Probably, however, a greater number of trisomics are actually viable in such a case, owing to the greater range of viability on the female side.

IX. INTERCHANGE TRISOMICS

Crossing-over between homologous interstitial segments located in non-corresponding regions of the differential segments of chromosomes of opposite complexes results in the type of segmental interchange which is a condition of half-mutant formation. This process is observable cytologically as a figure-of-eight and has been described both from *Oenothera biennis* (Darlington, 1931) and from ring-forming *Pisum* (Sansome, 1932; Sutton, 1935). Two new chromosomes are formed as a result of each such cross-over. These two new chromosomes can each occur only in one 7-chromosome combination which contains a full complement of pairing segments. Such interchange gametes may or may not be viable, depending upon whether the contained differential segments determine a vital or a lethal balance. Those gametes which *are* viable may conceivably be mated, not only with the two parental complexes, but also with 8-chromosome gametes, and in this way give rise to trisomics containing an interchanged chromosome.

The theoretically possible number of different interchange trisomics must be very large. For instance, in a plant with a ring of 14, there should be a maximum possible 49 for each viable interchange gamete, one corresponding to each different 8-chromosome gamete with which it can be mated. They should occur only very infrequently. For example, in *O. Lamarckiana* half-mutants occur approximately once in 1000 plants, and trisomics approximately once in 100, therefore one may expect to find one interchange trisomic in about 100,000 plants.

The maximum catenation and breeding behaviour of these trisomics also may be predicted. Certain of the expected maximum catenations are shown in Fig. 11, from which it will be seen that they are very variable and include the catenations characteristic of monomorphic trisomics.

As regards breeding behaviour, two classes may be distinguished. The one, in which interchange gametes are mated with 8-chromosome gametes of type A (p. 279), should give interchange trisomics which would

segregate two new types when selfed, namely, the half- and the full-mutant.

For example, the trisomic¹

A-gamete: $Ax_1B - By_1C - Cx_2D \quad Ex_3F \quad Gx_4H \quad Kx_5L \quad Mx_6N \quad Ox_7P$
 Interchange gamete: $By_1C \quad Dy_2E \quad FzA \quad Gx_4H \quad Kx_5L \quad Mx_6N \quad Ox_7P$

would be capable of forming X-complex gametes and interchange gametes. An X-complex gamete mated with an interchange gamete would give the half-mutant, while two interchange gametes would give the full mutant.

A second class of interchange trisomics should be formed as a result of making interchange gametes with gametes of types B, C and D (p. 280). These should normally segregate only the full mutant when selfed. The trisomic

B-gamete: $By_1C \quad Gx_4H - Hy_4K \quad Ly_6M \quad Ny_6O \quad Py_7A \quad Fx_3F - Ey_3D$
 Interchange gamete: $By_1C \quad Gx_4H \quad Kx_5L \quad Mx_6N \quad Ox_7P \quad AzF \quad Ey_3D$

would be capable of forming interchange gametes, but not X- or Y-complex gametes. Thus it can segregate the full mutant, but not the half-mutant when selfed. If the full mutant is not viable, then it will breed true.

Many of the interchange trisomics of the second class should have maximum catenations identical with those predicted for primary monomorphic trisomics. Should such a trisomic also breed true, it might be mistaken for a true primary monomorphic trisomic. It should be distinguishable from the latter by crossing with the parental diploid, for the half-mutant diagnostic of an interchange should occur among the progeny.

It is possible that there are some interchange trisomics which have arisen in the way suggested above among the many *Oenothera* trisomics which have not yet been studied either genetically or cytologically. They would be comparable with the tertiary trisomics of *Datura* (Blakeslee, 1930).

X. SUMMARY

1. As a result of four cytologically distinguishable varieties of non-disjunction in an *Oenothera* with a ring of 14 chromosomes, 49 different 8-chromosome gametic combinations may be formed which are possibly

¹ The differential segment of the interchanged chromosome *FzA* is written *z* as it may be composed partly of differential segment *X₁* of chromosome *Ax₁B* and partly of differential segment *y₃* of chromosome *Fy₃G*.

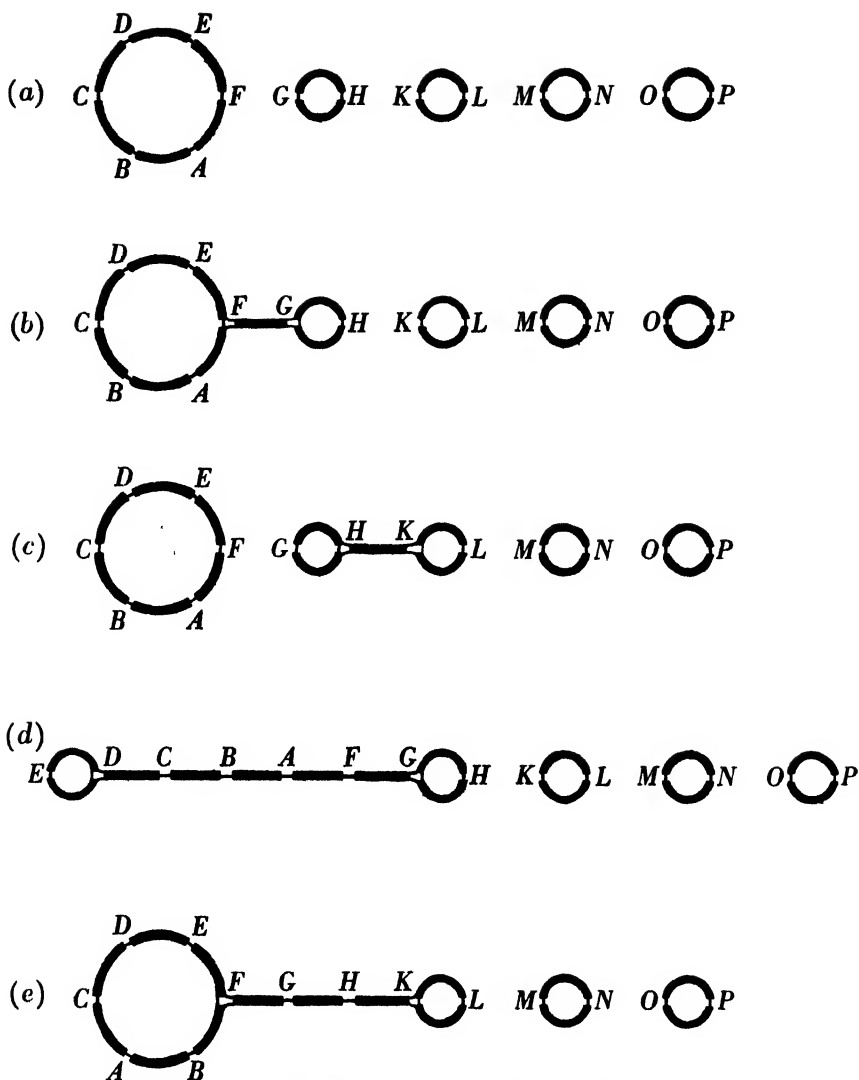


Fig. 11. Expected maximum catenations in some possible interchange trisomies derived from an interchange gamete (formed as a result of crossing-over between homologous interstitial segments located in non-corresponding segments of opposite complexes) mated with various 8-chromosome gametes.

The interchange gamete is:

$By_1C \quad Dy_2E \quad FzA \quad Gx_4H \quad Kx_5L \quad Mx_6N \quad Ox_7P.$

The 8-chromosome gametes are:

- (a) $Ax_1B \quad By_1C \quad Cx_2D \quad Ex_3F \quad Gx_4H \quad Kx_5L \quad Mx_6N \quad Ox_7P$ (Type A)
 (b) $Ax_1B \quad Cx_2D \quad Ex_3F \quad Fy_3G \quad Gx_4H \quad Kx_5L \quad Mx_6N \quad Ox_7P$ (Type A)
 (c) $Ax_1B \quad Cx_2D \quad Ex_3F \quad Gx_4H \quad Hy_4K \quad Kx_5L \quad Mx_6N \quad Ox_7P$ (Type A)
 (d) $Ax_1B \quad Cx_2D \quad Dy_2E \quad Fy_3G \quad Gx_4H \quad Kx_5L \quad Mx_6N \quad Ox_7P$ (Type B)
 (e) $Ax_1B \quad Cx_2D \quad Ex_3F \quad Fy_3G \quad Hy_4K \quad Kx_5L \quad Mx_6N \quad Ox_7P$ (Type B)

viable on the assumption that at least one representative of each pairing segment is a necessary condition of gametic viability.

2. Normally, 8-chromosome gametic combinations are only functional on the megaspore side, but may be mated with pollen of both complexes. In this way the 49 such combinations mentioned in (1) should yield a possible 98 different 15-chromosome zygotes. These should fall into 7 distinct cytological series of primary trisomics, one dimorphic and six monomorphic, with 14 forms in each. The latter should breed true, whilst the former should segregate the parent diploid when selfed. The maximum catenation of the dimorphic series should be an association of 15, forming a ring in which one link is doubled. The monomorphics should have a characteristic maximum catenation in which two ring pairs are joined by a chain consisting of one of the odd numbers from 1 to 13, plus a number (5-0) of free ring pairs, according to the particular series.

3. The maximum possible number of primary trisomics is determined for plants with chromosome rings of various sizes. These trisomics should have maximum catenations comparable to those of the trisomics derived from the plant with a ring of 14 chromosomes.

4. The number of primary trisomics of a heterogamous species is equal to the number of 8-chromosome gametes viable on the megaspore side, for these may only be mated in one way, namely, with the one complex which is functional on the pollen side. In *O. muricata*, a heterogamous species with a ring of 14, the full 49 8-chromosome combinations are possibly viable as megaspores, on the assumption that lethality of the *curvans* megaspores is due to the balance of differential segments present. On the alternative hypothesis that a single lethal gene is responsible for megaspore lethality, a maximum of 28 8-chromosome gametic combinations should be viable on the megaspore side. In the first case, a maximum of 49, and in the second a maximum of 28 primary trisomics are possible.

5. An analysis of the disjunctional arrangement in 65 nuclei of *O. laevigata* Bartl. var. *similis* Gates var. nov. which has a ring of 14, showed that 35 were normal and 30 non-disjunctional. The results indicated that there was a lower frequency of non-disjunction in complete rings as compared with the derived chains.

In *O. biennis* L., with rings of 6 and of 8, the frequency of non-disjunction as determined from 72 cells, was 3 per cent. in the former and 14 per cent. in the latter. The total of 17 per cent. is much lower than in *O. laevigata*, and the tentative conclusion is drawn that frequency

of non-disjunction increases with increasing number of chromosomes in the ring.

6. It is shown that interchange trisomics may be expected as a result of an interchange gamete mating with a viable 8-chromosome gamete. Such trisomics should occur approximately once in 100,000 plants in *O. Lamarckiana*.

I wish to acknowledge the valuable help and advice given me by Prof. R. R. Gates, F.R.S., under whose direction the work has been carried out. To Dr D. G. Catcheside, of this department, my thanks are also due for many stimulating and constructive suggestions. I also wish to thank Dr J. Henderson of the Mathematical Department, King's College, for advice.

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ARTIFICIAL THALLIUM MOULT IN SHEEP

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(With Plates VII-XI)

I. THE PROBLEM

THE object of this study was the possibility of artificially provoking physiological processes in individuals belonging to a race to which these physiological processes are not naturally proper, though characteristic of individuals of other races of different genetical constitution.

The study of the multiformity of morphogenetical reactions of organisms belonging to the same genotype (pluripotence) is an essential part of the problem of hereditary realization.

Up to the present the study of the phenomenon of pluripotence in the light of the analysis of the phaenogenesis of inherited characters (Haecker, 1925) has been limited almost exclusively to the study of morphological characters, i.e. to the final results of phaenogenesis. But besides morphological peculiarities one should, from a phaenogenetical standpoint, study also the processes characteristic of different races of animals.

As a concrete example we have chosen one of the rhythmically repeated morphogenetical processes, proper to practically all mammals, viz. the process of periodical (at any rate annual) renovation of the hairy covering, manifested by a more or less synchronous shedding of old hairs and a subsequent growth of new ones, i.e. moulting.

Among all the mammals man and merino sheep alone may be regarded as not being subject to a natural periodical moult (Iljin, 1932 c). The fine-woolled sheep (the merino, etc.) and the "coarse-woolled" sheep with mixed wool are a good example of a distinct and marked contrast in this phaenotypical character between two groups of animals with different genetical constitutions.

A periodical natural moult is absent from fine-woolled sheep throughout life. (Naturally this excludes pathological alopecia, or hair shedding due to mange, underfeeding, etc.) Bohm (1873) was the first to observe the wool growth during 9-10 years in unshorn sheep, i.e.

during almost their whole life period, finding its rate to decrease from year to year. In mixed woolled sheep on the contrary (Nathusius, 1880), a more or less sharply marked process of annual synchronous moulting is readily noticeable, accompanied in typical cases by successive detachment of the wool from the skin and its dropping off the body of the animal.

Hybrids between fine and mixed woolled sheep exhibit segregation of the character of the presence or absence of moulting. The presence of moulting is probably correlated with the heterogeneity of the wool cover, manifested by its differentiation into kemp, fine wool and some other types of wool fibres. The genetical nature of the presence or absence of a periodical change of wool, studied by Zorn (1919) and others, cannot yet be considered as completely analysed. Nevertheless, it is obvious that moulting, owing to distinct differences in its manifestation in different races, may be used as a very convenient subject for studying the problem of the *phaenogenesis of a process* proper to a certain race of animals.

Our chief problem was that of artificially inducing the moult in animals genetically devoid of that character, i.e. of provoking experimental moulting in naturally non-moulting animals. In other words, is it possible to induce in fine-woolled sheep and in their hybrids a process not proper to the race in question, though proper to another race with another genetical constitution?

II. MATERIAL AND METHODS

We used for our experiments (1) merino sheep of the Rambouillet type, (2) merino sheep of the new "Caucasus" type, (3) hybrids of mérinos-précoces with zigaya (sheep with the so-called semi-fine wool) (see Pl. VII, fig. 1 and Pl. VIII, fig. 1).

Experiments, carried out in 1932, included 843 merinos and hybrids treated with thallium (see Tables III and IV) and 507 control sheep under regular clinico-biological observation (the last group contained 118 merinos, 120 above-mentioned hybrids and 269 mixed woolled sheep of different races (see Table I).

Besides these there were control groups in which the condition of the wool covering only was studied. The total number of sheep under experiment and observation in 1932-3 was more than 2000. Experiments were carried out in Sovkhoses of Russia (Soviet State Farms), under different climatic conditions (Crimea, the South Ukraine, the North Caucasus

Moscow District and Moscow), and in different seasons (spring, summer, autumn, and winter).

As elsewhere explained (Iljin, 1932 *a* and 1932 *b*, etc.) the experimental production of moulting may be regarded as a temporary inhibition of the normal keratoplastic functions. To produce it we used various compounds of thallium (acetate, nitrate, carbonate, thallium alum, etc.). This action of thallium, known in medicine since 1898, was instigated by Sabouraud (1910) and others in their treatment of mycotic diseases of hair in man, as well as by Buschke and others on experimental alopecia (see Buschke and Peiser, 1931).

TABLE I

The number of sheep under regular clinico-biological observation (1932)

	Race	Treated with thallium (see Tables III and IV)	Untreated control
A.	Merino:		
	Rambouillet type	25	58
	New Caucasus type	112	60
		137	118
B.	Cross-breeds:		
	Mérinos-précoces-zigaya	155	120
C.	Mixed woolled:		
	Ukraine type "chushka-karakul"	—	90
	Russian "wallach"	—	20
	Crimean "malitch"	—	55
	Fat-rumped of Casakhstan	—	104
			269
	Total	292	507

The preparations we used in our experiments were administered to the animal in single doses *per os in boli*; later on by means of injection. The doses were calculated for each animal separately, 10–14 mg. per kg. body weight.

We apply the term *chemization of the organism* to the process of introducing into it the chemical preparations tested. Chemical compounds which induce experimental moulting we term *stimulators of moulting*.

III. EXPERIMENTAL MOULT IN FINE-WOOLLED SHEEP

Since the growth of wool fibres in fine-woolled sheep is in general synchronous (though its rate is sometimes different in different parts of the body), we supposed that the action of thallium, inhibiting the keratinization process in the growing hairs, might lead to temporary

and synchronous disturbances in the hair growth, a thinning or rupture of the hair fibre, and consequently to moulting.

The first experiments on the chemization of fine-woolled sheep (Iljin, 1932 c) showed the correctness of our suggestions. In 2 or 3 days after the introduction of thallium into the organism there may be noticed a visible diminution of the strength of the skin-hold of the fibres, i.e. a diminution of root strength, in comparison with that of the fibres of untreated animals.

Subsequently this diminution of root strength increases progressively. We characterize it according to 3-4 points system (see Table II). After a certain time period, different for different races (11-16 days at an average), the woolly covering becomes detached from the body of the sheep (see Pl. VII, fig. 2), ultimately resulting in complete shedding of the wool, i.e. a *spontaneous artificial moulting* (cf. the artificial moulting of Angora wool rabbits, Iljin, 1932 b; Rosanoff, 1933).

TABLE II
*The modifications of wool cover of merino sheep
after the thallium chemization*

Phases	Periods	
I. Degree of diminution of root strength of the hair	3- 4 days after the date of chemization	
II. " "	4- 6	" "
III. " "	7-12	" "
Spontaneous "shedding" of the wool staples	14-16	" "
The period of removal of the wool cover	11-13	" "
	and later	" "

After such moulting the skin becomes glabrous as if thoroughly shaved (see Pl. IX and Pl. X, fig. 1), differentiating it sharply from a sheep shorn in an ordinary way; the latter keeps on its body a large quantity of wool left there after shearing (see Pl. VIII, fig. 3).

In the majority of our fine-woolled sheep certain doses of thallium produced a moulting of almost the whole wool cover with the exception of acral parts, namely, the muzzle, the lower parts of the legs (Pl. VII, fig. 3; Pl. IX), sometimes a part of the breast (Pl. VIII, fig. 2) and the medial line of the belly (Pl. X, fig. 2).

Treatment with a dose of thallium smaller than the threshold dose for total moulting induces a shedding of fleece only on certain strictly localized areas of the body, on the back (Pl. X, fig. 4), on the back and sides (Pl. X, fig. 3), etc.

Further, we have found that it is possible to remove the wool cover *before* its spontaneous shedding. *This may be done by slightly pressing*

with the fingers the bases of the wool staples and successively taking off parts of the wool cover. It does not require any instruments (scissors, etc.) and is carried out by hands gently detaching the wool from the skin and taking off one part of the fleece after the other. The wool may be easily taken off as an undisrupted entire fleece in 15-7 or even 3-2 min.

The possibility of an artificial removal of the entire fleece in a short time has certainly a definite *practical interest*, and also allows of a more precise determination both of the degree of the diminution of the root strength and of the amount of wool obtained by means of the artificial moulting.

TABLE III

Chemization of the fine-woolled sheep

	Merino sheep, Rambouillet type (Soviet State Farm "Schmidt", Ukraine)	Merino sheep, new Caucasus type (Soviet State Farm No. 10, North Caucasus)	Total
Spring and summer, 1932			
No. of sheep under chemization (treated with thallium)	25	524	549
No. of moulting sheep	22	241	263
No. of chemized with lethal dose	10	62	72
No. of killed for anatomical research	—	24	24
Autumn, 1932			
No. of sheep under chemization (treated with thallium)	18	—	18
No. of moulting sheep	14	—	14
No. of chemized with lethal dose	8	—	8
No. of killed for anatomical research	—	—	—
Total no. of sheep under chemization			567
Total no. of moulting merino sheep			277

It stands to reason that besides the changes in the wool cover, the organism of a sheep, subjected to chemization, undergoes a number of definite physiological changes. Among the latter should be mentioned the toxic effects specific for thallium which were described in our other works.

The animal, denuded of its fleece by means of experimental moulting, grows a new uniform wool cover, and after a certain time acquires its usual aspect. The quality of the wool, taken off by means of moulting, as well as of that grown afterwards remains on the whole unchanged, with the exception of certain insignificant details in the root zone and of pointed tips described in other works from my Laboratory (Nicolajeff, Mochnieva and others).

By means of this method we obtained in 1932 an experimental moulting in 277 fine-woolled sheep (see Table III and Pl. XI, figs. 1, 2).

IV. EXPERIMENTAL MOULT OF HYBRIDS BETWEEN THE NON-MOULTING MERINOS AND THE SEMI-FINE-WOOLLED SHEEP

The hybrids under experiment were from complex crosses between merino-précoces and zigaya sheep, reared at the Crimea Soviet State farms (Sovkhoz) "Monay" and "Sai". Those chosen for the experiment owing to the quality of their wool were closely related to ordinary fine-woolled sheep (Pl. VII, fig. 1 and Pl. VIII, fig. 1), did not moult under natural conditions, and were therefore interesting subjects for experiment.

The result obtained by means of chemization was in the case of these hybrids in principle the same as in the case of pure-bred fine-woolled sheep (see Pl. VII, figs. 2, 3 and Pl. VIII, fig. 2), though it differed in certain details, chiefly the periods of the diminution of root strength in the wool and in the size of the necessary thallium doses.

In 1932 experimental moulting was obtained in 189 hybrids (see Table IV).

TABLE IV

Chemization of the cross-breed mérinos-précoces-zigaya

No. of sheep treated with thallium	276
No. of moulting sheep	189
No. of chemized with lethal dose	67
No. of killed for anatomical research	4

V. DISCUSSION

The natural difference which exists between the fine- and the "coarse-woolled" sheep with mixed wool in the process of the change of their wool cover and the presence of moulting may be experimentally obliterated. As a result of thallium treatment *we succeeded in provoking experimental moulting in 466 fine-woolled sheep and their hybrids* (see Table V), *which do not moult under natural conditions*, the process of moulting being proper to another group of races—the mixed woolled sheep.

TABLE V

The total number of fine-woolled sheep with experimentally induced thallium moulting (Exp. 1932)

Merino sheep, Rambouillet	36
Merino sheep "new Caucasus type"	241
Mérinos-précoces-zigaya	189
Total	466

The experimental moulting thus obtained, notwithstanding its similarity in a number of points to the moulting of mixed woolled sheep, differs

from the natural process, and the two cannot be considered as identical. At any rate the genetical constitution of fine-woolled sheep and their non-moulting hybrids does not, under certain conditions, prevent the occurrence of moulting, a regularly recurring physiological process.

I suppose that even with all possible restrictions the conclusion should be drawn that *the limits of the so-called "reaction norm"* for a given genotype are *much wider* than they seem to be according to experimental and theoretical data of contemporary genetics. This thesis found its corroboration in our former works on the phaenogenesis of the albino series pigmentation (Iljin, 1925, 1926, 1930, 1931).

Generally speaking phaenogenetical analysis should take into account the possibility of inducing physiological processes as well as morphological characters in norms not proper to the race in question (to the genotype in question), but proper to another race with another genetical constitution.

VI. SUMMARY

1. Fine-woolled races of sheep (merino and others) and the races of sheep with mixed wool are a good example of a marked contrast between two groups of animals genetically different as to the character of the presence or absence of a natural moult. In fine-woolled sheep no moult can be detected during their whole life period. In mixed woolled sheep the process of natural moult may be observed annually.

2. The hybrids of fine-woolled and mixed woolled sheep show segregation for the character of the presence or the absence of natural moulting. The presence of moult is evidently correlated with the heterogeneity of the wool cover, which is differentiated into kemp, fine wool and some other types of wool fibres.

3. By means of treatment with preparations of thallium an artificial, experimental moult may be provoked in fine-woolled sheep and their naturally non-moulting hybrids.

4. With experimental thallium moult the wool cover may be taken off the body of the sheep before its spontaneous shedding due to the chemization of the organism. Such removal of the wool is carried out by the naked hand gently detaching the wool from the skin and successively taking off one part of the fleece after the other. The wool cover is taken off as an entire unruptured fleece and the sheep becomes quite naked. The operation takes about 7-9 min., the minimal period being 2-3 min.

Later on the sheep which have undergone moulting begin to grow a new wool, and after a certain time acquire their usual aspect.

5. In this work the experimental thallium moult is considered as an antithesis to the process of wool growth and as the result of a temporary inhibition or stopping of keratoplastic functions, which leads to a disturbance in the synchronous growth of hair fibres—their thinning and breaking.

6. The experimental thallium moult is a convenient object for the study of the problem of the physiology of the wool cover development as well as of the general problems of phaenogenetics, especially in connexion with definite physiological processes.

7. The experiments described in the present paper were carried out on the Soviet State Farms (Sovkhoses) in the Crimea, the South Ukraine, the North Caucasus, the Moscow district and Moscow itself, on more than 2000 sheep.

VII. ACKNOWLEDGEMENTS

My extensive studies of the experimental thallium moult of sheep were carried out on a large number of sheep and in a relatively short time thanks to my numerous collaborators. For this my most hearty thanks are due to M. A. Rosanoff, P. B. Hofman, G. S. Uspienskaya, L. I. Levkovitsch, K. F. Levitskaja, M. G. Golysheva, A. D. Pshenichnikoff, M. E. Skorniakoff, T. A. Bedniakova, Prof. Dr A. I. Nikolajeff, H. J. Mochnieva, Prof. Dr B. K. Bohl, Prof. Dr S. S. Khalatov, A. V. Akuloff and others.

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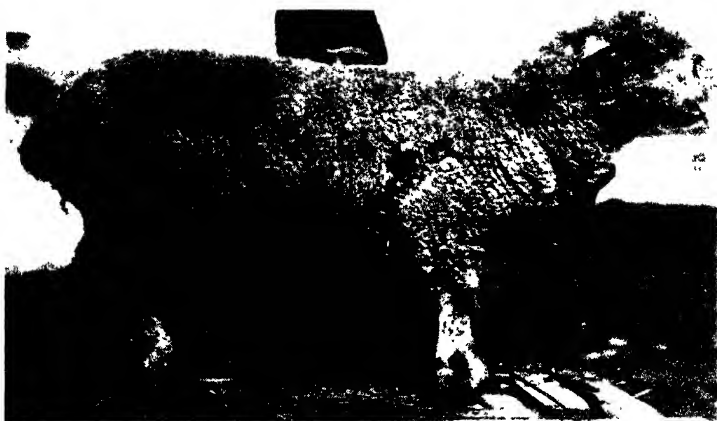


Fig. 1



Fig. 2



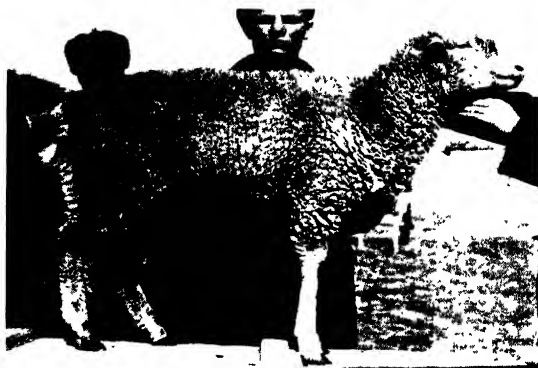


Fig. 1



Fig. 2



Fig. 3





Fig. 1



Fig. 2



Fig. 3



Fig. 4

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EXPLANATION OF PLATES VII—XI

PLATE VII

- Fig. 1. A fine-woolled ewe mérino-précoce-zigaya, untreated.
- Fig. 2. The same ewe after chemization with thallium. Note the detachment of wool on the shoulders.
- Fig. 3. The same ewe denuded of its fleece by means of artificial thallium moulting.

PLATE VIII

- Fig. 1. Mérino-précoce-zigaya, untreated.
- Fig. 2. Mérino-précoce-zigaya No. 128 after artificial thallium moulting.
- Fig. 3. A mérino-précoce-zigaya, untreated, sheared.

PLATE IX

Merino, new Caucasus type, after artificial thallium moulting.

PLATE X

- Fig. 1. Merino of Caucasus, after thallium moulting.
- Fig. 2. Mérino-précoce-zigaya No. 305, after thallium moulting.
- Fig. 3. Merino of Caucasus No. 182, after thallium moulting.
- Fig. 4. Merino of Caucasus No. 350, after treatment with a dose of thallium, smaller than a threshold of irritation dose.

PLATE XI

Figs. 1 and 2. A herd of treated sheep, denuded by means of thallium moulting, pasturing on Soviet State Farms; among denuded sheep there are several as yet unmoulted. (Chemization by Platon B. Hofman.)

THE CHROMOSOME MORPHOLOGY, SECONDARY ASSOCIATION AND ORIGIN OF CULTIVATED RICE

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(With Plate XII and Twenty-nine Text-figures)

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INTRODUCTION

PREVIOUS cytological investigations on the cultivated varieties of rice have shown that the diploid chromosome number is $2n=24$, but somatic chromosome morphology, meiotic chromosome behaviour and genetical evidence of the presence of duplicate, triplicate and multiple factors indicate that the constitution is not that of an ordinary diploid.

The cytology of rice, especially the meiosis, has been studied by Kuwada (1910), Selim (1930) and Nandi (1936, unpublished), but owing to its apparently diploid nature and the smallness of the chromosomes many important details were omitted. No complete analysis of the somatic complement of chromosomes has been made, and even the presence of satellite chromosomes was unknown until it was observed by Nandi (1936) as terminal deeply stained knobs attached to the nucleolus.

Secondary pairing was first observed by Kuwada (1910) in *Oryza sativa* L., from which evidence Lawrence (1931) tentatively concluded that *O. sativa* is derived from a form with 7 pairs of chromosomes, in common with the majority of the Gramineae. Yamaura (1933) suggested a possible origin of *O. sativa* by hybridization between two ancestral species with 5 and 7 chromosomes respectively, numbers characteristic of subfamilies nearly related to Oryzeae.

Further critical evidence is here presented to test the validity of these suggestions.

As no known species of *Oryza* has a lower chromosome number than $n=12$, the indirect method of approach to the problem by a study of secondary association and chromosome morphology was continued further. During the course of the investigation several significant facts have come to light, and the present paper suggests that the original ancestor of rice had a basic number of 5 chromosomes, and that the present number, $n=12$, is secondarily balanced. Sakai (1935) apparently subscribes to the same opinion.

MATERIAL AND METHODS

The following species and varieties were studied cytologically.

- (1) *O. sativa* L. $n=12$.
Kuruvi (Coimbatore).
Ratnagari wild rice (Coimbatore).
Black Puttu (Coimbatore).
Rajbhog (Assam).
Kaligira (Assam).
Kataktara, Dacca no. 2 (Bengal).
Indrasail (Bengal).
Punjab 41, Mushkan (Punjab).
Punjab 28/28, Sathi (Punjab).
Konan-sen (South China).
Omati (Japan proper).
Tanko-hoira (Formosa).
Banto-Sinrika (Japan proper).
- (2) *O. officinalis* Wall. $n=12$.
- (3) *O. minuta* Presl. $n=24$.

The seeds were sown in pots during the years 1934 and 1935 in the greenhouse at the genetical laboratory, Regent's Park, London. Root tips were fixed in Benda fixative, with excellent results. Pollen mother cells were fixed partly in medium Flemming but mainly in Navashin's fixative (Maeda, 1930), the latter giving the better results. All slides were stained by Newton's iodine gentian-violet technique. Root tips and anthers were cut at 12–14 μ .

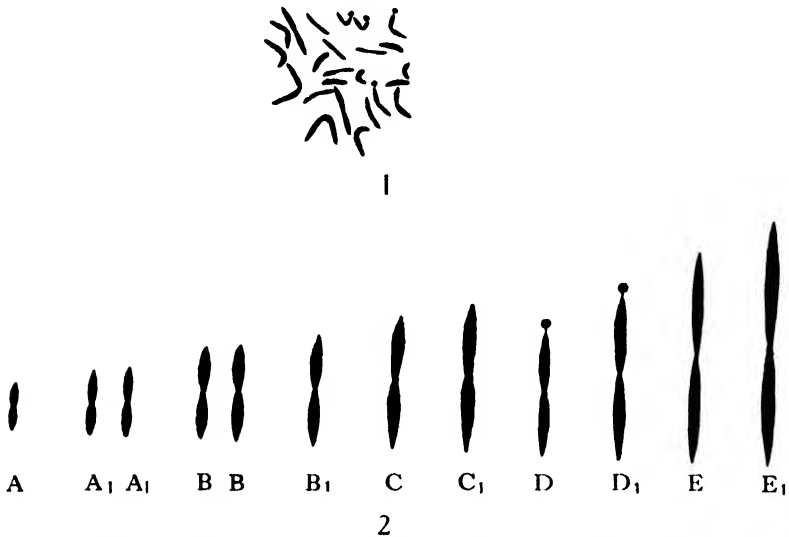
SOMATIC CHROMOSOME COMPLEMENTS

In material well fixed with Benda, the chromosomes were generally well spaced out and slender, so that their size variations and morphology

could be worked out fairly accurately. The chromosomes of *O. sativa* are rather small, the longest being about 2.8μ and the smallest 0.7μ long.

The existence of a long pair of chromosomes with well-marked knobs, and the presence of only two such chromosomes observed by Avdulov (1931) made it possible for him to suggest that the basic number in *O. sativa* L. is $n=12$.

In the present work the characteristic morphology of the somatic chromosomes and the size relationship was studied in considerable detail from a large number of metaphase plates, in order to classify each type of chromosomes and the number of times they occur.



Text-fig. 1. Tanko-hoira. Somatic metaphase from root-tip of *O. sativa*, $2n=24$. ($\times 3200$.)

Text-fig. 2. Semi-diagrammatic representation of the haploid genome indicating the two sets of chromosomes from the two five-paired species with duplication of one chromosome in each set.

The somatic metaphase plate (Text-fig. 1) shows that the longest pair is characterized by a median constriction which typically gives a V shape; the apex of the V being always turned towards the centre of the equatorial plate. The shortest chromosome pair is distinctly shorter than the rest of the chromosomes. Two other pairs are well marked by small, but distinct, satellites attached by a slender thread to the proximal end of the chromosomes. There is a definite size difference between these two pairs of satellited chromosomes, one pair being longer than the other. Other intermediate comparable pairs with size differences exist which make it possible to classify the 24 chromosomes of *O. sativa* L. into ten

types. Of these ten types of chromosomes, eight are present twice and two types are present four times, making a total of $2n = 24$ in the diploid complex. These observations were verified from studies of meiotic chromosomes, where the size differences and types of chromosomes are more marked.

These results are depicted semi-diagrammatically in Text-fig. 2, which represents the haploid genome of *O. sativa* L. As is shown in the figure, the ten types of chromosomes fall into two groups of five types each, there being one exactly similar chromosome in each group. The members of these two groups are designated: *A*, *BB*, *C*, *D*, *E* and *A*₁*A*₁, *B*₁, *C*₁, *D*₁, *E*₁. It is evident that *A* is definitely shorter than *A*₁*A*₁, *B*₁ is slightly longer than *BB*. There is very little difference between *C* and *C*₁. *D*, with a satellite, is definitely shorter than *D*₁ also with a satellite. *E*₁ is distinctly longer than *E*. It is also quite obvious that *A*₁ and *B* occur twice. The haploid complex of *O. sativa* may then be regarded as composed of two different five-paired sets belonging to two different species in which two chromosomes were duplicated, the two species concerned differing somewhat in chromosome morphology.

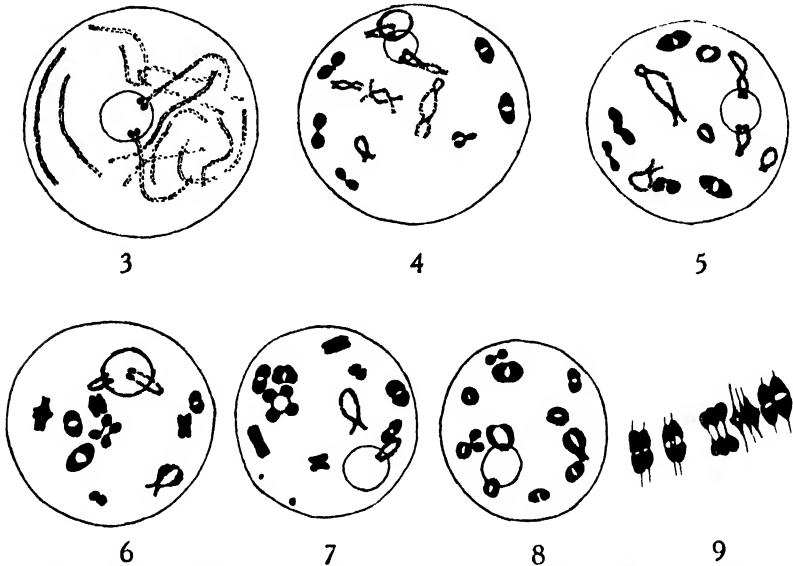
MEIOSIS

The chromosome behaviour of the different varieties of *O. sativa* L. studied is so similar that the following account is applicable to all.

Previously (Nandi, 1936) two chromosomes were found at early prophase attached to the nucleolus by terminal knobs, which bodies were referred to as responsible for the orderly organization of the nucleolus in the previous telophase. Sometimes a second pair of chromosomes was also found attached to the one or two nucleoli, but it was uncertain whether this pair was really organically connected with the nucleolus or was lying over it. Further studies have shown definitely that two pairs of chromosomes with terminal satellites are attached to the nucleolus from the early prophase stage until the time of disappearance of the nucleolus at prometaphase (Text-figs. 3-8). Sometimes, as shown in Text-fig. 7, one pair of chromosomes was found attached to the nucleolus, the other pair being detached from the nucleolus probably by the microtome knife.

In the microphoto (Pl. XII, fig. 1), two pairs of chromosomes at zygotene with deeply stained satellites are clearly evident, and are found attached to the nucleolus. The presence of four satellite chromosomes is further confirmed by studies on the somatic metaphase chromosomes. Navashin (1912), in *Galtonia*, first discovered a pair of satellite chromosomes

attached to the nucleolus. According to his definition satellites are "nucleosomes" found on the surface of the nucleolus, which are later picked up by fine threads sent out from two chromosomes, which thus become satellited. This view has been adopted by Sorokine (1924, 1929), Babcock & Navashin (1930), Senjaninova (1926) and Heitz (1928).



Text-fig. 3. Omati. A pollen mother nucleus with twelve pairs of zygotene threads. Note the spiral nature of the threads. Two pairs of chromosomes are attached to the nucleolus by terminal satellites. ($\times 2300$.)

Text-figs. 4-7. Kaligira. Pollen mother nuclei at early diakinesis showing differential condensation of the twelve bivalents and various chiasmata. Note the longest pair with three chiasmata. Two unequal pairs of satellite chromosomes are attached to the nucleolus or nucleoli. Text-fig. 7 shows one pair of chromosomes attached to the nucleolus, the other pair being displaced by the microtome knife. Note one quadrivalent with four similar-sized chromosomes. ($\times 2300$.)

Text-fig. 8. Kaligira. Late diakinesis where terminalization of chiasmata is nearly complete. ($\times 2300$.)

Text-fig. 9. Konan-sen. Side-view metaphase I showing secondary association of bivalents, 3 (2) + 2 (3), maximum association. N.B. Chromosomes and chromosome groups separated laterally. ($\times 2300$.)

Numerous cases of the presence of satellite chromosomes have been observed in plants. There are also a few cases in which no satellites have been observed. Heitz (1931) states that all plants must have satellites, haploid with one, diploid with two, triploid with three and tetraploid with four, and so on. The absence of satellite chromosomes, as has been reported in a few species, is therefore not due to their real absence but due to the difficulty in obtaining good material for their detection. In

most cases satellites have been described at metaphase. In the first meiotic metaphase the morphological features of the chromosomes cannot be seen clearly owing to the contraction and condensation of the chromosomes which transform a long chromosome of diakinesis into a spherical body as observed in rice, a conformation unsuitable for the observation of satellites. The same is to some extent true for somatic metaphase. I am therefore quite in agreement with the statement of Heitz (1931) that all plants must have satellites, and that their number must always coincide with the number of genomes present in a species. Satellites can therefore be described as minute portions of chromosomes joined by a constriction at the proximal end of the chromosome, and they are of vital importance to the cell, their function being to organize the nucleoli. But satellites may sometimes change their position on the chromosome as has been observed by Darlington (1929) in *Rhoeo*, where they are interstitial. If satellites were "nucleosomes" in the sense of Navashin (1912), found on the surface of the nucleolus, they should certainly always take up a terminal position and never an interstitial position on the chromosome.

It is very difficult to get good stages of early prophase where the chromosome threads are so delicate as in rice. A few cells were found in which the fixation was critical, and twelve pairs of zygotene threads could be counted easily (Text-fig. 3). In some of the threads the spiral structure of the chromosomes is quite evident. The "chromomeric" appearance of the leptotene and zygotene stage, which has been described by various authors, does not represent the true structure of the chromosomes. Studies on plants with big chromosomes, as in *Hosta* (Nandi, unpublished) and *Hyacinthus* (Naithani, unpublished), have clearly shown that the superficial chromomeric appearance is due to the minor coiling present in leptotene and zygotene threads.

The zygotene threads then appear to shorten and increase in thickness, resulting in the pachytene stage, which leads to diplonema when the separation of the homologous threads begins. In contrast to the synapsis, which starts from the ends of the chromosomes, the separation starts at the middle, no doubt from the region of spindle-fibre attachment. As this happens, each of the apparently single threads which paired at zygotene becomes double, resulting in a quadripartite condition. The gradual shortening and thickening of the threads lead to early diakinesis, when it can often be seen (Text-figs. 4-5) that a chromatid from one chromosome exchanges partner with another chromatid of its homologue. Consequently these chiasmata may be regarded as the

obvious cytological evidence of crossing-over between chromosomes. The chiasmata undergo terminal movement during the coiling of the chromatids into major spirals, such spirals running parallel in the two chromatids.

Further contraction of these bivalent chromosomes leads to complete terminalization, with the result that the two chromosomes of a bivalent are held together by terminal connexions into rod- or ring-shaped bodies. But some of the chiasmata do not terminalize fully, the bivalents retaining interstitial chiasmata at diakinesis and metaphase I.

The types of bivalent observed at early diakinesis and metaphase are shown in Text-figs. 4-9. It is evident that there are distinct differences in the sizes and variable consequence of variable position of chiasmata of the twelve bivalents. A description of the chromosome types has already been given in the analysis of somatic chromosomes. Identification of the chromosomes in somatic plates made it possible to follow them through late prophase stages of meiosis, and the configurations of bivalents could be interpreted as follows:

(1) The longest pair of chromosomes with median constriction usually form three chiasmata, and by metaphase these chiasmata are completely terminalized to give a ring bivalent with two terminal chiasmata.

(2) Medium-sized chromosomes with median insertion region may form a chiasma in each arm, which terminalizes by metaphase I to give a ring bivalent with two terminal chiasmata, or the chiasma in one arm may be incompletely terminalized.

(3) Small chromosomes form a chiasma in one arm, and by metaphase I the chiasma completely terminalizes resulting in a rod bivalent with one terminal connexion.

The frequencies of terminal and interstitial chiasmata at early diakinesis and metaphase were determined from fifteen nuclei. Interstitial chiasmata are very frequent at early diakinesis, and fall off rapidly until metaphase I. The mean number of chiasmata also diminishes concurrently from 1.7 at early diakinesis to 1.5 at metaphase. This demonstrates the effects of terminalization. Further, Table I and Text-figs. 4-9 show that as meiosis proceeds the total number of chiasmata decreases, but the proportion of the chiasmata that are terminal increases. This analysis of the chiasmata in rice supports the statement of Newton & Darlington (1929) that "in plants and animals with smaller chromosomes where the existence of chiasmata has usually been ignored, the superficial appearance is merely the result of extreme condensation of the loops formed at diplotene". It also demonstrates, as Darlington (1929) has shown

for *Hyacinthus*, that the number of chiasmata varies with the length of the chromosomes, the highest number observed in the longest bivalent of rice at diakinesis being three and the smallest number being one chiasma in the small chromosomes.

At metaphase I, as already at diakinesis, the size differences of the bivalents are noticeable, and a variable number of bivalents are secondarily paired. The theory of secondary association of meiotic chromosomes originally put forward by Darlington (1928) and systematized by Lawrence (1931) implies that the chromosomes showing this kind of affinity are phylogenetically related to each other. Secondary association is therefore "intimately connected with allopolyploidy" (Lawrence, 1931).

Secondary pairing can be used as a measure of distant relationship between the chromosomes present in a polyploid, and it will be so used

TABLE I
Analysis of chiasmata in Oryza sativa L.

No. of nuclei	No. of pairs of bivalents	Total no. of chiasmata	Mean no. of chiasmata per bivalent	No. of interstitial chiasmata	No. of terminal chiasmata	No. of terminal chiasmata per bivalent	Terminalization coefficient	Highest no. of chiasmata in any bivalent	Stage
15	180	300	1.7	135	165	0.91	0.55	3	Early diakinesis
15	180	270	1.5	45	225	1.2	0.83	2	Metaphase

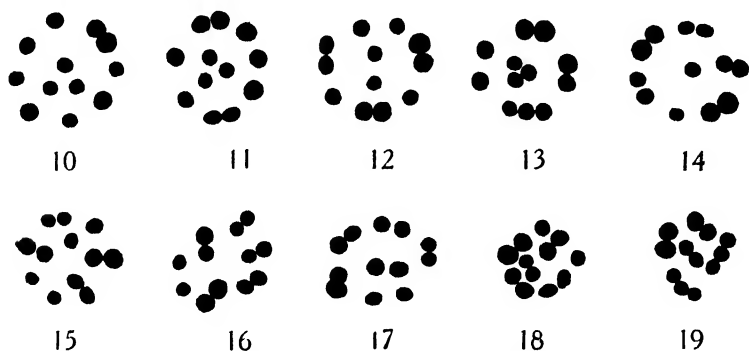
in determining the basic composition of the chromosomes in *Oryza sativa* L.

Analysis of complete side-views at metaphase is difficult owing to the secondarily paired chromosome groups sometimes lying perpendicularly to the plane of the microscope field. Text-fig. 9 shows twelve bivalents from a first metaphase, the bivalents have been separated laterally in the drawing, care being taken not to separate those bivalents which were seen lying in juxtaposition. It is evident that paired bivalents in any one group show similar numbers of chiasmata, either two or one terminal or interstitial.

The various types of secondary association observed in polar view (Text-figs. 10-19) and microphoto (Pl. XII, figs. 3, 5) are summarized in Table II *a, b*.

Although a considerable number of cells were examined, six groups of two bivalents secondarily associated were never observed. The number of secondary associations per metaphase plate ranges from one to five, the mode being four (Table II *a, b*). Groups of three bivalents are

commonly found. The maximum association observed consists of two groups of three bivalents and three groups of two bivalents in a plate (Text-fig. 19). Therefore the basic chromosome number is $b^*=5$. Sakai (1935) also regards 5 as the primary basic number of *O. sativa*, from which the present number 12 had been derived by secondary polyploidy. The maximum secondary association observed by Sakai is the same as I have observed, namely two groups of three and three groups of two.



Text-figs. 10-19. Banto-Sinrika. Polar views, metaphase I, showing varying degrees of secondary association of bivalents and chromosome arrangement. ($\times 2300$.)

Text-fig.	Association of bivalents	Chromosome arrangement.
		N.B. Groups of secondarily associated bivalents are taken as one body
10	(1) + 1 (2)	8, 3
11	8 (1) + 2 (2)	7, 3
12	6 (1) + 3 (2)	7, 2
13	2 (1) + 2 (2) 2 (3)	5, 1
14	2 (1) + 5 (2)	6, 1
15	4 (1) + 4 (2)	7, 1
16	2 (1) + 5 (2)	7, none inside
17	4 (1) + 4 (2)	6, 2
18	2 (1) + 5 (2)	6, 1
19	3 (2) + 2 (3)	5, 1
	maximum association	

Anaphase is normal, there being no lagging chromosomes but secondary association is maintained (Text-fig. 20).

It is known that secondary association may be more marked at metaphase II (Lawrence, 1931; Müntzing, 1933). In rice the secondary associations of univalents are more pronounced at metaphase II (Text-figs. 21-22, Table II *b*). Two groups of three univalents, two groups of two univalents and two separate univalents are clearly seen in the microphoto (Pl. XII, fig. 5). Similar groupings of univalents persist at

* The letter *b* is used in this paper as a symbol for the basic number as suggested by Gates (1935).

anaphase II (Text-fig. 23). Tetrad formation is regular in all the forms and irregularities are rare.

TABLE II

(a) *Types of secondary associations at metaphase I in rice*

Nos. of secondary associations	Nos. of bivalents in association			No. of cells	Totals
	1	2	3		
1	10	1	—	4 }	6
1	9	—	1	2 }	
2	8	2	—	6 }	8
2	6	—	2	2 }	
3	6	3	—	8 }	14
3	4	1	2	4 }	
3	5	2	1	2 }	
4	4	4	—	13 }	19
4	2	2	2	6 }	
5	2	5	—	9 }	12
5	—	3	2	3 }	
					59

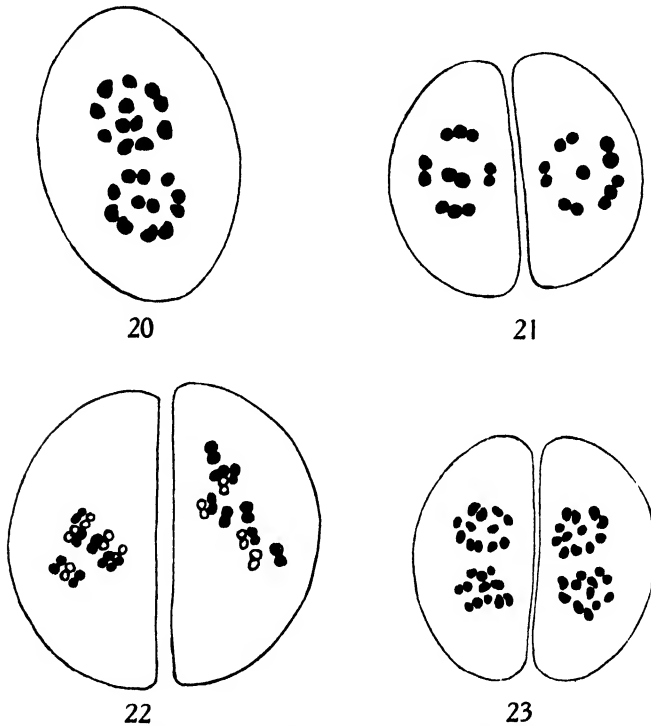
(b) *Types of secondary associations at metaphase II in rice*

Nos. of secondary associations	Nos. of univalents in association			No. of cells	Totals
	1	2	3		
1	10	1	—	6 }	9
1	9	—	1	3 }	
2	8	2	—	8 }	13
2	7	1	1	3 }	
2	6	—	2	2 }	
3	6	3	—	10 }	16
3	5	2	1	4 }	
3	4	1	2	2 }	
4	4	4	—	18 }	25
4	2	2	2	7 }	
5	2	5	—	12 }	16
5	—	3	2	4 }	
					79

CHROMOSOME ARRANGEMENT ON THE METAPHASE PLATE

The question whether the chromosomes on metaphase plates are arranged purely by chance or are dependent upon some force has not been clearly answered as yet. I shall attack the problem from two angles, viz. (1) the behaviour of metaphase chromosomes in relation to the floating magnet theory of chromosome arrangement (Kuwada, 1929), and (2) the behaviour of the secondarily associated bivalents or univalents at metaphase in relation to the floating magnet theory.

It is remarkable that in *Oryza sativa* L. a few plates show no secondary association at metaphase I (microphoto, Pl. XII, fig. 4), the twelve bivalents being arranged more or less regularly with three chromosomes in the centre and nine on the periphery corresponding to the stable form of



Text-fig. 20. Kuruvi. Polar view anaphase I showing secondary association of univalents. ($\times 2300$.)

Text-fig. 21. Kuruvi. Polar view metaphase II showing secondary association of univalents, the left-hand one with 3 (2) + 2 (3) maximum association, the right-hand one with 1 (1) + 4 (2) + 1 (3). ($\times 2300$.)

Text-fig. 22. Tanko-hoira. Metaphase II in side view with persistence of secondary pairing (chromosomes at top focus shown in outline and bottom focus in black). ($\times 2300$.)

Text-fig. 23. Tanko-hoira. Anaphase II in polar view showing regular distribution of univalents and secondary pairing still persistent. ($\times 2300$.)

arrangement of floating magnets. This non-association of otherwise associated bivalents seems to be due to the corresponding bivalents not lying near each other. The mutual attractions of the widely separated corresponding bivalents are not sufficient to permit of secondary association. In that case secondary association must be dependent upon chance, so that proximity of the two or three bivalents at diakinesis or prometa-

phase will be the deciding factor in the development of secondary association.

In case (2) the groups of secondarily paired bivalents or univalents are taken as single units. It is very significant, as shown in Table III, that the groups of secondarily paired bivalents or univalents are found to act as if they were single bodies, arranging themselves on the metaphase plate in the same relation as floating magnets. The stable form of arrangement has a relatively high frequency, 76.1 per cent. as compared with the other forms of arrangement 23.5 per cent. As is observed (Text-fig. 12) when there are three pairs of bivalents secondarily associated making a total of nine bodies, the number of inner bodies is two, but when only two pairs of bivalents are secondarily associated, making

TABLE III
*Arrangement of secondarily associated chromosomes
in relation to floating magnets*

Stable arrangement					Unstable arrangement				
No. of chromosomes in the periphery	No. of inner chromosomes	Secondary association involved	No. of cases	%	No. of chromosomes in the periphery	No. of inner chromosomes	Secondary association involved	No. of cases	%
9	3	None	3		8	4	None	2	
8	3	1	10		8	1	3	3	
7	3	2	14		8	2	2	5	
7	2	3	9		6	2	4	4	
7	1	4	13		7	—	5	3	
6	1	5	12		6	—	5	2	
5	1	5	7		5	—	5	3	
4	1	5	2					22	23.5
			70	76.1					

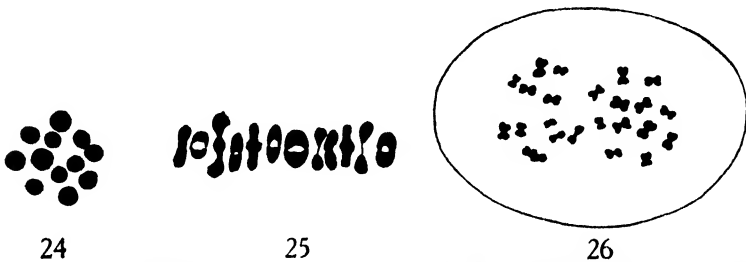
a total of ten, the number of inner chromosomes is three (Text-fig. 11), both these forms resembling the stable form of arrangement of floating magnets with nine and ten bodies respectively. This condition shows that the mechanism of chromosome distribution on the metaphase plate is not disturbed by secondary association, and that, when the secondary association is present, the groups of paired bivalents act as single bodies and arrange themselves in accordance with a corresponding number of floating magnets. Gates (1909) showed that the chromosomes act at diakinesis and telophase like floating bodies which mutually repel each other

Alam (1936) in *Eruca* and *Brassica* found the stable form of arrangement of chromosomes on the metaphase plate more or less distorted by the effects of secondary pairing. The irregularities in the arrangement observed in some cases by him and by the author may be explained as

due to the action of fixatives, the chromosomes at that stage being in oscillating movement. Naturally they would be fixed in various stages of movement before the final distribution of the metaphase chromosomes is attained.

Oryza officinalis Wall.

The number of somatic chromosomes in this species is 24. They seem larger than those of *O. sativa* L. At metaphase I of meiosis pairs of bivalents are seen secondarily associated (Text-fig. 24). The degree of secondary association is less marked than in *O. sativa* L., and some



Text-fig. 24. *O. officinalis* ($2n=24$). Polar view metaphase I with some of the bivalents secondarily associated. ($\times 2300$.)

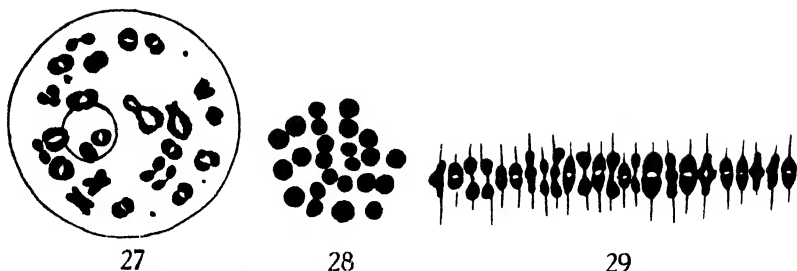
Text-fig. 25. Same in side view showing three interstitial chiasmata, three rod bivalents and six ring bivalents. Note the chromosomes are definitely bigger than *O. sativa*. ($\times 2300$.)

Text-fig. 26. Anaphase I in polar view, all the chromosomes showing median constriction and the double nature of the univalents. ($\times 2300$.)

plates were found in which there was no secondary association present. A complete side view of metaphase I is shown in Text-fig. 25. Text-fig. 26 shows anaphase I in polar view, the double nature of each chromosome being indicated by a median constriction.

Oryza minuta Presl.

The number of somatic chromosomes in this species is 48. At diakinesis there are regularly 24 bivalents (Text-fig. 27). At metaphase secondary association is of frequent occurrence. Sufficient cells were not available to find out the types of secondary association. Text-fig. 28 shows the heterotypic metaphase in polar view, and Text-fig. 29 a complete side view. Here we can approximately distinguish 24 big and 24 small chromosomes. *O. minuta* Presl. is a distinct, stable and fertile species which has perhaps originated by hybridization between *O. officinalis* \times *O. sativa*, followed by chromosome doubling. It is therefore an allo-octoploid species.



Text-fig. 27. *O. minuta* ($2n=48$). Diakinesis with twenty-four bivalents of different size and shape, of which four are attached to the nucleolus. ($\times 2300$.)

Text-fig. 28. Metaphase I in polar view showing secondary association.

Text-fig. 29. Metaphase I in side view. Note the size differences of the chromosomes of different shape. ($\times 2300$.)

DISCUSSION

The origin of rice

The evidence, historical and prehistorical, shows that the cultivation and domestication of rice is very ancient. In India, from the Aryan invasion it has the Sanskrit name "Vrihi arunya". Roschevitz (1931), from a botanico-geographical investigation, concludes that the centre of origin of rice was in Tropical Africa where the largest number of the species of *Oryza* are found; the centre of greatest diversity of rice varieties is, however, India, the condition of that country favouring mutation under cultivation. According to Reynier (quoted in De Candolle, 1883), rice came to India from China. Whether the origin and cultivation of rice first started in India, China or Africa, it is certain that in 2800 B.C. the Emperor Chenming in China instituted a ceremony at which rice was sown as one of the five principal crops (De Candolle, 1883). In the opinion of the author the centre of origin of cultivated rice can hardly be determined from the evidence at present available.

Wild rice as a weed is widely distributed among the rice fields in swampy regions all over Central Africa, India and Indo-China, the countries where the cultivation of this cereal is most ancient and intensive. Roschevitz (1931) suggests that *O. officinalis*, *O. minuta* and a few other species of wild rice characterized by a perennial habit, short ligule, glabrous spikelets, short anthers, small seeds and brittle rachis and nearly allied to cultivated rice, were the progenitors of some of the cultivated rice varieties. This is doubtful since, although *O. officinalis* has the same chromosome number as *O. sativa*, the chromosomes of the former are larger than those of the latter. Moreover, breeding experi-

ments¹ have shown that the hybrid between these two species is completely sterile. On the other hand, *O. minuta* ($n=24$) is an allo-octoploid species and so will not hybridize with *O. sativa*. The evidence shows that, however long we grow the wild rices such as *O. officinalis* and *O. minuta*, they retain from year to year their characteristics and do not change into cultivated rice.

There are important specialized characters which are found only in cultivated rice. These are the annual habit, a restricted period of flowering, a non-brittle rachis and large seeds, all these characters no doubt selected by human agency.

According to the opinion of the author the immediate wild progenitor of *O. sativa* is still unknown and perhaps no longer exists.

O. sativa is usually regarded as a diploid, but the cytological data support the genetical evidence that it is polyploid in nature.

The somatic chromosomes have been classified as described above into ten types, two groups of five types each, which differ in certain details. In the somatic complement having 24 chromosomes there can be recognized two members of eight types and four members of two types. This certainly indicates that the haploid genome of the present *O. sativa* is composed of two original five-paired species belonging to two different ancestral genomes in which two chromosomes were duplicated. Otherwise if the chromosomes of *O. sativa* had been derived from the reduplication of a primary set of six chromosomes we should have encountered four chromosomes of each type represented four times in the set and should have observed a maximum of six pairs of secondarily paired bivalents.

As has been described before, two types of chromosome association occur in the apparently diploid species of rice—first the primary association of the chromosomes forming 12 bivalents and a variable secondary association of these bivalents at first metaphase, or of the 12 univalents at second metaphase, the maximum association being two groups of three and three groups of two making a total of five, which is therefore the basic number of *Oryza*.

O. sativa thus agrees with *Pyrus* (Darlington & Moffett, 1930), *Dahlia Merckii* (Lawrence, 1931), *Brassica napus* (Catcheside, 1934) in being

¹ It may be mentioned here that in the cross made by Ramiah (1936), one of the species mentioned as *O. latifolia* is in fact *O. officinalis*. Seed material of this species, kindly sent by Ramiah and grown by the author, has been determined from Kew Gardens. *O. latifolia*, a species with tall habit, broad leaves and hairy ligules, is endemic to South and Central America.

secondarily balanced, but with the difference that *Oryza sativa* is allo-tetraploid, whereas the above-mentioned cases are probably auto-polyploids.

In that case the present haploid chromosome formula of *O. sativa* may be written as follows:

$$\begin{array}{ll} A & A_1, \\ B & B_1 \\ C & C_1 \\ D & D_1 \\ E & E_1 \end{array} \quad (n=12)$$

Mr Ramanujam allows me to state that the somatic chromosome number observed in the nearly related genus *Zizania* is $2n=30$ (*Z. aquatica*). It is probably an allo-hexaploid species based on $n=5$. No other species in the Oryzeae has been cytologically examined, but the evidence makes it predictable that the primitive number in this group is 5.

In *Dahlia Merckii*, a secondarily balanced allo-tetraploid species, it is possible to point to the related primary allo-tetraploid species within the genus, but in *Oryza* no species occurs with the primary tetraploid number $2n=20$. In the absence of such evidence the following hypothesis shows the possible course of events in the evolution of *O. sativa*.

Suppose the original species with a haploid set of 5 chromosomes, respectively *A*, *B*, *C*, *D*, *E*, produces by structural changes another species whose chromosomes would be A_1 , B_1 , C_1 , D_1 , E_1 . A cross between these two species would very likely be sterile. A similar case is seen in *Nicotiana Bigelovii* \times *N. suaveolens* (Goodspeed & Clausen, 1927), where there is one bivalent out of 24 chromosomes, and in *Raphanus sativus* \times *Brassica oleracea* (Karpechenko, 1927) in which the 18 chromosomes usually fail to pair, there being occasionally one bivalent.

Related species with unrelated chromosome numbers are of widespread occurrence in the plant kingdom, as in *Crepis* (Babcock & Cameron, 1934), *Iris* (Simonet, 1934), *Brassica* (Morinaga, 1928-31), and *Primula* (Bruun, 1932). In *Crepis* the haploid chromosome numbers 3, 4, 5, 6, 7, 8 are most common. All these numbers are probably secondarily balanced, derived from a primary number. This shows that reduplication of single chromosomes is involved in species formation.

Matsuura (1935) finds secondary association of two bivalents in *Tricyrtis*, $n=13$, and *Dicentra*, $n=8$, from which he concludes that *Tricyrtis* and *Dicentra* are secondarily balanced diploids derived from

ancestral forms having haploid sets of 12 and 7 units respectively. Similarly, Meurman (1933) in *Acer* observed a strong secondary pairing of two bivalents at metaphase I and suggests that the haploid chromosome number $n=13$ in *Acer* spp. is a secondarily balanced basic set derived from an ancestral set of 12 units.

Under such circumstances it is conceivable that viable aneuploid gametes with $n=6$ arose through meiotic irregularities in a cross between the two species concerned, and that these gave rise to a plant having 12 chromosomes. Chromosome doubling either somatically as in *Primula Kewensis* (Newton & Pellew, 1929) and *Nicotiana glauca* (Clausen, 1928) or gametically as in *Raphanus-Brassica* cross (Karpechenko, 1927, 1928), *Digitalis ambigua* \times *D. purpurea* (Buxton & Newton, 1928), *Nicotiana rustica-paniculata* (Lammerts, 1931) could produce a secondarily balanced allo-tetraploid, with $n=12$. It would be fertile, vigorous and constant, through autotetraploidy.

Haploid derivatives of rice are stated to show lack of synapsis, namely that the haploid chromosome set does not contain any wholly homologous chromosomes. I actually find well-marked secondary association, viz., two groups of three univalents and three groups of two univalents in the figures of haploid rice published by Ramiah *et al.* (1934). This implies that chromosomes, which descended from originally homologous chromosomes, even though they are too altered to pair primarily, reveal their affinity by pairing secondarily into definite groups. I have concluded that in the haploid genome of *O. sativa* one chromosome, e.g., A_1 , from one ancestral species is duplicated and another chromosome, e.g., B , from the other ancestral species is also duplicated. Naturally we should expect two bivalents in the haploid rice. Morinaga (1934) has observed one or two bivalents in haploid plants of rice. Similarly it may be expected that two quadrivalents could be formed in the (apparently) diploid rice. I have actually seen one true quadrivalent, having the four chromosomes similar, in one out of 200 nuclei observed. This rarity of quadrivalent formation is no doubt due to the competition in chiasma formation which leads to bivalents.

The available evidence on the meiotic behaviour of haploid rice further confirms that *Oryza sativa* is a secondarily balanced allo-tetraploid originated through hybridization between two 5-paired species with duplication of two chromosomes due to meiotic irregularities in the hybrid followed by a subsequent doubling of the chromosomes, thus giving $n=12$ as the fundamental number for the existing species of *Oryza*. Consequently the chromosome sets of *O. sativa* may be regarded

as not representing the reduplication of any basic set, i.e., it is not an auto-tetraploid.

As in the Rosaceae, such an unbalance has produced a distinct group Pomoideae with basic number 17 from a primary number of 7 (Moffett, 1931), similarly the genus *Oryza* may have arisen by such an altered chromosome constitution from *Zizania* with a basic number of 5. This change in balance must have resulted in a new type of plant different from its progenitor in morphological characters.

The chromosome constitution of *Oryza sativa*, as analysed above, is a sufficient basis for its complicated genetical results. The production of numerous races of rice probably has a relation to the increase in chromosome numbers, since the latter would favour greater opportunity for mutation and accumulation of reduplicated factors.

SUMMARY

1. Chromosome numbers were determined in fourteen varieties of *Oryza sativa* L. The observations show the apparent diploid number $2n=24$ in all the forms, there being no variation in the number. *O. officinalis* Wall., $2n=24$, has larger chromosomes than *O. sativa*. *O. minuta* Presl., $2n=48$, is an allo-octoploid species which has probably originated by hybridization between *O. officinalis* \times *O. sativa*, followed by a chromosome doubling.

2. A detailed study of *O. sativa* reveals the existence of ten types of chromosomes which fall into two groups of five types each. Of these ten types of chromosomes eight are present twice and two types are present four times, making a total of $2n=24$ in the diploid complex.

3. During the heterotypic prophase two unequal pairs of chromosomes with terminal satellites remain attached to the nucleolus until the nucleolus disappears completely at pro-metaphase.

4. Pairing at diakinesis was found to be by chiasmata. Chiasma frequency was analysed in the pollen mother cells of fifteen nuclei. It is found that as meiosis proceeds the total number of chiasmata decreases, but the proportion of total chiasmata that are terminal increases from diakinesis to metaphase.

5. The twelve bivalent chromosomes at early diakinesis differ in size and shape. The longest pair shows three chiasmata and the smallest one chiasma. Exceptionally one true quadrivalent with four chromosomes indistinguishable in size has been observed.

6. Secondary pairing occurs amongst the bivalents at metaphase I or univalents at metaphase II. The minimum number of groups of

bivalents or univalents is five, which is therefore the primary chromosome number in *Oryza*. Of these five groups when secondary pairing is at maximum, two are made of three and three of two bivalents or univalents each. *O. sativa* is therefore a secondary balanced polyploid.

7. In the heterotypic and homotypic metaphase it is found that the groups of secondarily paired bivalents or univalents act as single bodies and in the majority of the cases arrange themselves in agreement with the stable form of arrangement of the corresponding number of floating magnets.

8. Somatic chromosome analysis, meiotic chromosome behaviour, together with the published evidence from haploid rice indicate that *O. sativa* is a secondarily balanced allo-tetraploid which originated through hybridization between two different five-paired species in which two chromosomes were duplicated, probably due to meiotic irregularities in the hybrid. This followed by a subsequent doubling of the chromosomes attained the secondary balance of $n=12$, the present existing number in *O. sativa*.

9. It is obvious that allo-polyploidy and the establishment of a secondary basic number are important factors in evolution.

Cyto-genetical data combined with taxonomical studies of the genus *Oryza* which are in progress will no doubt throw further light on the basic genetic relationships and origin of species.

It is my sincere desire to express my sincerest gratitude to Prof. R. R. Gates, D.Sc., LL.D., F.R.S., under whose supervision this work was done, for his valuable suggestions and unfailing kindness. The author also gratefully acknowledges the valuable advice and criticism of Dr D. G. Catcheside.

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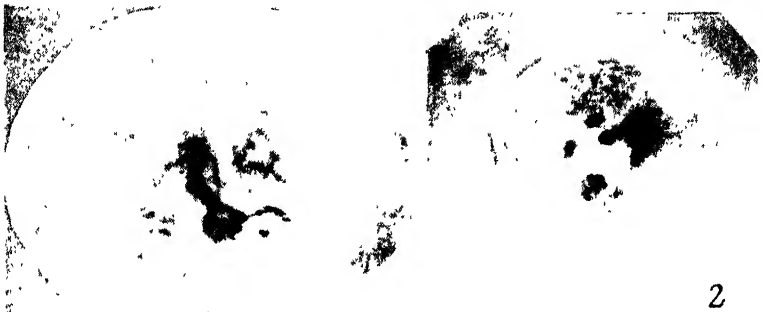
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EXPLANATION OF PLATE XII

Figs. 1-5. Microphotographs of the pollen mother cell division in *Oryza sativa*.

- Fig. 1. Omati. Zygonema, two pairs of chromosomes attached to the nucleolus by deeply stained terminal satellites. ($\times 1150$.)
- Fig. 2. Kalijira. Diakinesis showing a quadrivalent with four similar chromosomes. ($\times 1150$.)
- Fig. 3. Banto-Sinrika. Metaphase I with five groups of two bivalents secondarily associated and two bivalents separate. Note the size variations of the bivalents. ($\times 3500$.)
- Fig. 4. Omati. Metaphase I, the bivalents free from any secondary association, with nine chromosomes in the periphery and three in the centre. ($\times 1150$.)
- Fig. 5. Omati. Metaphase II. The left-hand plate showing two groups of three and two groups of two univalents secondarily associated, two univalents being free. The right-hand plate in side view and out of focus. ($\times 1150$.)



1

2



3



4



5

A BLACK-BLUE DUTCH RABBIT

By JAMES N. PICKARD

(Institute of Animal Genetics, University of Edinburgh)

(With One Text-figure)

CASES of mammals exhibiting both the recessive and the dominant members of a pair of allelomorphic characters in their coats are by no means common but have occasionally been reported in both rabbits and mice. An instance of this condition was recorded by the writer⁽³⁾ in which a rabbit showed several brown and several Angora patches in an otherwise short, black coat. Breeding tests proved the rabbit to be genetically a short-coated black animal, heterozygous for Angora and brown. No tendency to transmit the mosaic condition to its offspring was found.

The rabbit with which the present report deals was a buck, born on 22 March 1932, and presented to the Institute when about a month old by the breeder, to whom our thanks are due. The animal was one of a nest of six, the remaining five all being typical black and white Dutch. The parents were both black Dutch, the result of at least 20 years' continuous line breeding from one original pair of Dutch, a blue buck and a black doe, during which period the only outcross that had taken place was through the purchase some 12 years previously of an unrelated tortoiseshell Dutch buck which was used for one season. No fresh stock had been introduced into the stud for over 12 years prior to the birth of the rabbit now reported.

The rabbit was a fairly well-marked exhibition type black Dutch buck remarkable in that from the centre of the back down the side and immediately adjacent to the black rump, a band of blue (dilute) pigment was shown, gradually narrowing over the left shoulder and completely covering the left foreleg apart from a white tip to the foot (cf. Fig. 1). The rabbit was retained in the rabbitry until the summer of 1935 when it died. During its existence it was used fairly extensively for breeding, and the following results were recorded.

In twelve litters produced from five black Dutch does, known to be heterozygous for blue, this buck sired fifty-three youngsters, of which thirty-eight were black and fifteen blue. These matings showed a slight excess of blues above the expectation, supposing the buck to be genetic-

**Fig. 1**

ally a black, heterozygous for blue, which hypothesis further breeding tests substantiated.

The black-blue buck was also mated to three different blue does, which produced in six litters thirty youngsters of which sixteen were black and fourteen blue, a slight excess of the dominant colour.

Upon some of his daughters reaching maturity, nine of them, five blues and four blacks, were back-crossed to him, the former producing in ten litters forty-two youngsters of which twenty were blacks and twenty-two blues, a slight excess above the expectation of blues being found. From his four black daughters, he sired nineteen youngsters, all blacks excepting two blues. In this mating the doe K was later proved to be homozygous for black, whilst during her breeding life doe J produced no blues and therefore was also probably homozygous for black. Does L and M were by the present matings proved to be heterozygous for blue.

None of these 144 youngsters sired by the buck was a mosaic. It appears, therefore, conclusive that the effect of the condition had not extended to the gonads, as the proportions of his black and blue youngsters were very closely in accord with the normal Mendelian expectation.

A somewhat similar mosaic reported by Castle⁽¹⁾ appeared in the F_2 of a Dutch Angora cross, and was a black-blue Dutch. In this case, however, the original tricolour sired others like himself, as also did his tricolour son. In no other instance of mosaicism reported in rabbits or mice has there been any marked tendency for the condition to be transmitted, so it appears to be exceptional for the gonads to be involved.

In the case now reviewed the condition was not transmitted, and the most simple explanation, and one which meets the case, would be that at some time during development the dominant black gene was lost from a particular cell, thus allowing the recessive blue to become expressed in the affected area.

Extension of pigmentation into the normally white area is an exceedingly common failing in exhibition Dutch rabbits. These "runs" of colour are most frequently at one side and extend over the shoulder, the exact amount varying in different individuals from a small spot to an almost complete exclusion of white on one or both shoulders. Although an examination has not been completed, it seems probable that some specific gene or genes control the markings in this position and their extent, which in exhibition specimens must be absent.

In every case of mosaicism reported by Castle, as in the instance now reviewed, the exhibition of the recessive character is in a position which in typical animals is white, and in consequence the area controlled by the

gene or genes now postulated must have been affected. Similarly it has also been found in several reported instances of mosaicism in mice, that the areas normally white are most frequently affected. It seems, therefore, for some reason the genes controlling the development of colour in the normally white areas are least stable and most easily affected in cases of mosaicism.

In the cases of mosaic mice reported by Pincus(4), inbreeding had been carried on for several generations, whilst that recorded by Dunn(2) was the result of crossing two inbred strains. Castle(1) does not state in his report whether his Dutch and Angoras had been inbred for some generations prior to their having been crossed, but this is probably the case with the Dutch at least, as they "were descended from a single pair". In the present case inbreeding or close-line breeding had been undertaken for 20 years. In view of this, continued inbreeding or outcrossing of inbred strains appears to show a tendency to affect the somatic stability of a stock.

SUMMARY

A mosaic black-blue and white Dutch rabbit is reported. The condition was not transmitted to any of his 144 offspring. The possibility of the presence of genes controlling pigmentation in normally white areas is discussed, as also is the tendency for somatic instability being increased through inbreeding.

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BREEDING RECORDS

Black-blue buck mated to heterozygous black does

Litter	Doe's letter	Blacks	Blues	Total
1	A	4	1	5
2	B	4	—	4
3	C	2	2	4
4	D	2	3	5
5	B	5	—	5
6	A	5	1	6
7	C	3	2	5
8	E	3	1	4
9	D	4	—	4
10	B	3	1	4
11	D	2	1	3
12	E	1	3	4
		38	15	53
<i>Expectation</i>		<i>39.75</i>	<i>13.25</i>	

Black-blue buck mated to blue does

Litter	Doe's letter	Blacks	Blues	Total
1	F	2	4	6
2	G	3	2	5
3	F	1	5	6
4	H	4	1	5
5	F	3	—	3
6	H	3	2	5
		16	14	30
<i>Expectation</i>		<i>15</i>	<i>15</i>	

Black-blue buck mated to blue daughters

Litter	Doe's letter	Blacks	Blues	Total
1	N	2	2	4
2	P	2	3	5
3	Q	—	3	3
4	R	3	1	4
5	S	2	—	2
6	P	4	2	6
7	R	1	5	6
8	S	1	3	4
9	Q	3	2	5
10	S	2	1	3
		20	22	42
<i>Expectation</i>		<i>21</i>	<i>21</i>	

Black-blue buck mated to black daughters

Litter	Doe's letter	Blacks	Blues	Total
1	J	3	—	3
2	K	4	—	4
3	L	5	1	6
4	J	4	—	4
5	M	1	1	2
		17	2	19

INHERITANCE OF TAIL TIP PIGMENTATION IN THE HOUSE MOUSE

BY HANS GRÜNEBERG

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IN this laboratory, two pure lines of mice are kept which differ in the pigmentation of the tail tips. One of these is a black agouti pure line (*CBA*) in which most animals have the whole tail pigmented down to the very tip. The other line (black non-agouti), obtained from Dr L. C. Dunn, shows a large percentage of animals with unpigmented pink tail tips.

In a count of the agouti line, 79 out of 81 animals were found to have entirely dark tails with occasionally occurring small indistinct areas which are slightly less densely pigmented. One specimen had a minute trace of white on the tail tip, about $\frac{1}{2}$ mm. long, and one specimen showed a pink tip about 3–4 mm. in length. Longer unpigmented areas, up to half the tail length, occur rarely in this stock, though no such animal happened to be alive when the count was made. There is thus a small percentage ($2 \pm$) of light-tailed mice in the otherwise dark-tailed agouti line.

In the non-agouti line 52 out of 79 mice had pink tail tips ranging from 1 mm. to about one-third the total length of the tail (still longer tail tips are common), 10 mice had a little white tip of less than 1 mm., and 17 individuals had entirely dark tails. Thus in this line, about 78 per cent. of the animals have at least a short pink tip on their tails, while the resting 22 per cent. lack it.

There is no indication that segregation for presence or absence of light tail tips takes place in the non-agouti line. The stock appears to be homogeneous as regards these characters, there being no correlation between parents and offspring examined as regards their tail tips.

In order to get some information about the genetical basis of the difference between the two lines, a cross was made. An F_1 of 27 animals was raised; 26 of them had dark tails, and 1 had a tiny white tip. So this corresponds well to the behaviour of the dark-tailed agouti line. Pigmented tail tip seems therefore to be dominant over pink tip. However, there seemed to be slightly more mottling on the tails of the F_1 individuals. Although I cannot be too positive about this point, it might be an indication that dark tail is not completely dominant.

344 *Inheritance of Tail Tip Pigmentation in the House Mouse*

In F_2 the following figures were obtained:

	Agouti		Non-agouti		n
	Dark tip	Light tip	Dark tip	Light tip	
Observed	61	26	22	8	117
Expected (9 : 3 : 3 : 1)	65.8	21.9	21.9	7.3	116.9

The single factor ratios are: 87 agoutis to 30 non-agoutis and 83 animals with dark tail tips to 34 specimens with light tips. There is thus a segregation for the tail tips approaching a 3 : 1 ratio, suggesting that one single recessive factor is responsible for the light tail tips.

In a back-cross of F_1 animals to the non-agouti pure line these figures were obtained:

	Agouti		Non-agouti		n
	Dark tip	Light tip	Dark tip	Light tip	
Observed	39	27	32	36	134
Expected (1 : 1 : 1 : 1)	33.5	33.5	33.5	33.5	134

These figures do not differ significantly from 1 : 1 : 1 : 1 ratio expected on the assumption that light tail is a recessive segregating independently of the agouti series of allelomorphs. There is a slight deficiency of recombination types as compared with the original types (59 : 75), but the difference is insignificant ($\chi^2 = 1.910$ for $n = 1$). Moreover, classification in these experiments is not absolutely reliable, there being a certain number of animals where scoring may have been faulty. No great weight should therefore be attributed to minor numerical inconsistencies.

In view of the fact that light tail tips do not occur in all animals of the non-agouti line, but only in about 78 per cent., it is surprising that no deficiency of light tail tips was found in the F_2 or the back-cross. A few light tips occur also in the agouti line, but this would hardly counter-balance the normal overlaps in the non-agouti line. It may therefore be that a certain number of heterozygotes showed the character, and that dark tail is only incompletely dominant. This suggestion may also explain the somewhat unexpected result of a back-cross of F_1 animals to the agouti pure line. Forty-one animals were raised; 33 of them showed entirely dark tails, as expected, but 8 had light tips of varying length. This is more than would be expected within the agouti pure line. The difference is significant ($\chi^2 = 7.036$ for $n = 1$), but in view of possible mistakes in scoring, this should be taken with caution. So possibly some of the 8 light tailed animals are due to incomplete dominance, and incomplete dominance may also explain why light tail tips appeared in rather more than expected numbers in F_2 and the other back-cross.

Genes affecting the pigmentation of the tail tip are very common in tame mice and are also not rarely found in free living mouse populations. An analysis is only practicable in highly inbred material.

SUMMARY

In an agouti pure line, most of the animals have dark tail tips. In a non-agouti pure line, about 78 per cent. of the animals have light tail tips. The difference is due to a single gene pair, dark tail tip being dominant though possibly not completely so.

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THE EFFECT OF VARYING GENE DOSAGE ON ALEURONE COLOUR IN MAIZE¹

By M. M. RHOADES²

(With One Text-figure)

INTRODUCTION

It has not been possible, with the exception of a few outstanding cases (Sturtevant, 1925; Mangelsdorf & Fraps, 1931; Lindstrom & Gerhardt, 1926), to obtain reliable quantitative data on the change produced in a genetic character by varying the dosage of the major gene or genes determining the nature of that character. Data of this type are of value. A hypothesis which seeks to explain the role of a gene in ontogeny must take into account the change produced by varying the number of times it is present.

The aleurone tissue of the maize kernel offers exceptionally favourable material for the study of gene dosage and interaction. Several hundred seeds are produced on a single ear under the same environment. The aleurone is triploid in nature, since it arises from the union of a haploid sperm with two haploid polar nuclei. The seed on one ear may be of several genotypes. It is possible to vary the dosage of any allele from one to three in disomic strains, and the use of the proper primary trisome allows the dosage to range from one to five. In spite of these advantages there has been little work on the effect of gene dosage on aleurone colour because none of the previously known aleurone characters have been adapted to the obtaining of quantitative data. This paper presents a study of the effect of gene dosage on a new aleurone character in maize.

Four major genes are known to be involved in the development of aleurone colour in the maize kernel (East & Hayes, 1911; Emerson, 1918; Jenkins, 1932). These are A_1 , C , R , and A_2 . The A_1 locus is in chromosome 3, the C locus in chromosome 9, the R locus in chromosome 10

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and the A_2 locus in chromosome 5. Aleurone colour is not formed if the recessive allele of any one of the four primary loci is homozygous, i.e. at least one dominant allele of all four loci must be present. The alternative of purple or red aleurone colour depends upon the $Pr\ pr$ genes, purple being dominant to red. A ratio of 3 coloured to 1 colourless seed is obtained in F_2 when one of the four primary loci is heterozygous; a 9 : 7 ratio when two loci are heterozygous, etc.

In a selfed ear of Black Mexican sweet corn obtained from Dr L. F. Randolph, aleurone colour segregated into 12 self-coloured : 3 dotted : 1 colourless seeds. The Black Mexican line had been maintained for several generations by sibbing. The occurrence of colourless aleurone in a strain of corn homozygous for all the dominant alleles for aleurone



Fig. 1. Photograph of crown of maize seed showing the dotted aleurone character. The genetic constitution of this seed is $a_1a_1a_1DtDt$ and it is homozygous for the other dominant genes necessary for aleurone colour.

colour was unexpected, but more surprising was the appearance of a new dotted aleurone character. The dots or spots of colour were distributed apparently at random over the aleurone layer. They were small and fairly uniform in size (Fig. 1).

The colour of the dots is either purple or red depending upon the $Pr\ pr$ constitution. The intensity of the colour is greatest in the central portions of the dots with a gradual fading towards the surrounding colourless tissue.

The 12 : 3 : 1 ratio of the original ear was correctly interpreted as the segregation of one of the four primary genes, to give 12 self-coloured : 4 non-self-coloured (a 3 : 1 ratio), and a dominant gene which interacted with the recessive primary gene to give dots of aleurone colour in three-fourths of the non-self-coloured class. The segregating primary aleurone

factor proved to be A_1a_1 . The dominant gene interacting with a_1 to give the dotted character has not previously been reported. It has been designated **Dt dt**, the **Dt** allele giving dots of aleurone colour with a_1 , whereas **dt** does not.

The conclusion that the segregation of A_1a_1 occurred following a mutation of A_1 to a_1 seems unescapable, because the genetic residuum of the Black Mexican line was unchanged and out-crossing would have been detected. The **Dt** gene was thought, at first, to have arisen by mutation in the same gamete in which the mutation from A_1 to a_1 occurred, but tests of sister plants showed the **Dt** gene to be segregating in the Black Mexican strain, its presence not having been previously detected because the strain was homozygous for the dominant alleles of genes producing aleurone colour.

TABLE I

Interaction of Dt with the different aleurone genes

Genotype	Aleurone colour
a_1CRA_2DtPr	Purple dots on colourless background
a_1CRA_2Dtpr	Red dots on colourless background
A_1cRA_2Dt	Colourless—no dots
A_1CrA_2Dt	Colourless—no dots
A_1CRA_2Dt	Colourless—no dots
a_1CRA_2dt	Colourless—no dots
A_1CRA_2Dt	Strong self-colour—no dots
$a_1^pCRA_2Dt$	Pale self-colour—no dots
$A_1a_1CRA_2Dt$	Strong self-colour—no dots
$a_1^pa_1CRA_2Dt$	Pale self-colour with dots
a_1cRA_2Dt	Colourless—no dots
a_1CrA_2Dt	Colourless—no dots
a_1CRA_2Dt	Colourless—no dots

Dt interacts with a_1 to give dots of aleurone colour only if all the remaining three primary genes have at least one dominant allele present. **Dt** interacts in this manner only with a_1 . Seeds homozygous for a_2 or for c or for r , the remaining three genes in each case being present in dominant form, have colourless aleurone in the presence of **Dt**. The interaction of **Dt** with the different aleurone genes is given in Table I.

The linkage group to which the **Dt** gene belongs has not been established. **Dt** is, however, independent in its inheritance of the A_1 , C , R , A_2 and Pr loci, genes all affecting aleurone colour, as well as of the Lg_1 , Su , Y and F_1 loci.

RELATION BETWEEN DOSAGE OF a_1 AND NUMBER OF ALEURONE DOTS

There are four alleles at the A_1 locus. Two of these, A_1 and A_1^b , produce deep-coloured aleurone; a_1^p produces pale-coloured aleurone and a_1 produces colourless aleurone. Nothing is known concerning the interaction

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of A_1 and Dt , as dots of colour, if formed, are invisible in the deep aleurone colour produced by A_1 . Seeds homozygous for a_1^P have no dots of colour in the presence of Dt , although the intensity of colour in the dots on seeds of a_1Dt constitution is greater than in the pale aleurone colour and they would be visible if present. However, seeds carrying a_1^P and one or two a_1 alleles have dots of aleurone colour which are clearly evident on the pale-coloured background. Therefore it is possible to determine the effect of different dosages of a_1 on the aleurone dots by varying the ratio of the a_1 and a_1^P alleles. As the aleurone is triploid the dosage of genes in it may be varied from one to three. The dosage of a_1 may be varied while that of Dt is held constant, or the dosage of Dt may be varied while that of a_1 is held constant.

Crosses of $a_1a_1^PDtDt \times a_1a_1dtdt$ were made to determine the effect of three a_1 alleles as compared with one a_1 allele on the aleurone dots. Two classes of seed were produced on the same ear having the following constitution:

- (1) $a_1a_1^Pa_1^PDtDtdt$ = pale aleurone with dots,
- (2) $a_1a_1a_1DtDtdt$ = colourless aleurone with dots.

These two genotypes occur at random on the ear. Their environment is identical. They have the same genetic residuum except for the possibility of segregating genes linked with a_1 . The other aleurone factors were present in a homozygous dominant condition. The two classes, therefore, may be considered as differing only in that one of them has one a_1 and the other three a_1 alleles.

The number of dots of aleurone colour was determined on each seed under a low-power binocular after the seed coat had been removed to increase the accuracy of counting. Counts on the seeds in both classes were made independently by two investigators. The agreement was always close. Counts were made on the kernels from four ears, approximately fifty seeds of each class being counted from each ear. No appreciable increase in the reliability of the mean number of dots per seed was obtained by counting more than fifty seeds.

The average number of dots per seed of $a_1a_1a_1$ constitution was about three times as great as on seeds of $a_1a_1^Pa_1^P$ constitution which is the same ratio as the number of a_1 alleles in the two classes. The data from one ear are given in Table II with a statistical analysis. The data from the three other ears also were treated statistically but the deviations from a 3 : 1 ratio were not significant. The ratio of the mean numbers of dots per seed in the two classes for all four ears was 3.1 : 1.0.

The effect of three a_1 alleles as compared with two a_1 alleles was studied by analysing the data obtained from the cross $a_1a_1DtDt \times a_1a_1^pdt dt$. The two classes of seed produced have the following genotypic constitution:

- (1) $a_1a_1a_1^pDtDt dt$ = pale aleurone with dots,
- (2) $a_1a_1a_1DtDt dt$ = colourless aleurone with dots.

Data on the two classes were obtained from three ears. The ratio of the average number of dots on seeds of $a_1a_1a_1$ constitution to the average number on seeds of $a_1a_1a_1^p$ constitution was 3 : 2. This is the same ratio

TABLE II

Numbers of aleurone dots on 55 seeds with three a_1 genes and 55 seeds with one a_1 gene from an ear (Pedigree 2946-6 \times 2944-1) of the cross $a_1a_1^pDtDt \times a_1a_1dt dt$

$a_1a_1a_1DtDt$ class				$a_1a_1a_1^pDtDt$ class			
39	54	47	79	24	15	19	10
111	88	65	59	20	25	16	37
55	73	45	64	26	27	16	18
68	43	48	39	37	8	23	12
35	35	75	64	32	42	16	21
75	53	63	106	24	43	21	1
66	47	47	47	32	20	27	23
57	55	75	46	20	23	17	2
52	54	56	79	14	18	17	16
15	12	75	22	31	21	23	29
71	81	54	43	19	40	10	17
51	52	113	55	18	13	18	22
53	39	53	29	12	23	24	14
57	75	51	—	38	47	18	—

Mean = 58 dots per seed

Mean = 21.8 dots per seed

$t = 1.6442$. (108 D/F). Not significant.

as the dosage of the a_1 allele in the two classes of seed. Data from one ear are given in Table III. The ratio of the averages for the two classes of seed for the three ears was 3.06 : 2.0. The deviations from a 3 : 2 ratio for the individual ears were not significant.

The above data indicate that the effect on the dotted aleurone character of increasing the number of recessive a_1 alleles, as determined by the number of dots, is additive or linear.

In obtaining the data on the a_1 gene the number of Dt genes was held constant. Crosses therefore were made in which the number of a_1 genes remained constant while the Dt gene varied in dosage. The following cross was made reciprocally: $a_1a_1DtDt \times a_1a_1dt dt$. When the dotted parent was the female the seeds were all $a_1a_1a_1DtDt dt$. In the reciprocal cross, however, the seeds were $a_1a_1a_1Dt dt dt$. These crosses are exact reciprocals and were made between lines originating from the ear on which the dotted character first appeared so that they have much

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the same genetic make-up. It is important to emphasize that these reciprocal crosses should be made between lines related as closely as possible because the seed produced on two different plants is to be compared. The dosages of any genes modifying the number of aleurone dots would be different in reciprocal crosses between unlike lines, as we are dealing with triploid tissue in which two-thirds of the heredity is contributed by the female parent. The mean number of dots per seed in the a_1a_1DtDt class was about four times that for seeds of

TABLE III

Numbers of aleurone dots on 76 seeds with three a_1 genes and 60 seeds with two a_1 genes from an ear (Pedigree 2676 \times 2511-a) of the cross $a_1a_1DtDt \times a_1a_1^pDtdt$

a_1a_1DtDt class						$a_1a_1^pDtdt$ class					
6	25	27	19	8	20	15	3	18	18	10	
11	29	29	21	30	20	8	11	19	10	9	
8	17	3	26	23	29	16	10	25	10	7	
25	11	19	37	21	14	6	1	5	8	10	
13	25	7	32	17	4	7	5	18	3	15	
21	8	20	23	7	20	9	19	3	11	7	
12	9	19	38	14	17	9	6	19	13	—	
5	29	9	8	6	16	31	13	12	7	—	
27	15	36	16	14	12	14	10	9	5	—	
5	11	17	16	7	15	4	11	10	9	—	
10	9	37	12	20	16	15	14	6	13	—	
22	26	20	10	14	—	3	13	5	7	—	
33	8	8	23	20	—	9	14	14	20	—	

Mean = 17.6 dots per seed

Mean = 11.0 dots per seed

$t = 0.8532$. (134 D/F). Not significant.

a_1a_1DtDt constitution. Data on one reciprocal cross are presented in Table IV. The data are not as extensive as might be desired but the deviation from a 4:1 ratio of the means for the two kinds of seeds is not significant. The ratio of the means of the two seed classes for the three reciprocal crosses approximated 4:1, the actual ratio being 4.2:1.0. There is no *a priori* reason for assuming theoretically that the ratio of these two means be exactly 4:1. The data from the three ears ranged about this value so it was arbitrarily chosen. The ratio of dosages of **Dt** in the reciprocal crosses was 2:1. However, the effect on the number of aleurone dots was not additive as was the case for the a_2 gene. There were four times as many dots when two **Dt** genes were present as there were where only one **Dt** gene was present.

Data on the effect of three **Dt** genes can be obtained from a selfed plant homozygous for the **Dt** gene. Obviously these data cannot be compared with those for two and one doses of **Dt** unless the different

lines used in obtaining the three different dosages of **Dt** were very closely related. The only data available for three **Dt** genes came from seed obtained by selfing a plant in a line derived from the original dotted ear. It is, therefore, related to the other lines, but some differences in genetic constitution could and undoubtedly did exist. The data should be considered with discretion. The average number of dots per seed on this selfed ear was 185.0. The average numbers of dots on seeds with two and one **Dt** genes were 45.4 and 10.4, respectively. The data on the three **Dt** genes are, perhaps, not strictly comparable with the others, but it is possible to state, considering the data on the three classes of seeds,

TABLE IV

*Numbers of aleurone dots on individual seeds from reciprocal crosses, one cross yielding seeds with two **Dt** genes and the other yielding seeds with one **Dt** gene*

Pedigree 2951-9 × 2949-8 a₁a₁DtDt × a₁a₁dt dt a₁a₁DtDt seeds				Pedigree 2949-8 × 2951-9 a₁a₁dt dt × a₁a₁DtDt a₁a₁Dt dt seeds			
55	77	55	37	8	16	30	16
29	69	19	28	4	12	15	6
45	69	32	49	7	8	7	10
20	42	35	36	8	3	8	3
17	57	56	—	14	11	10	—
20	52	52	—	12	17	9	—
78	16	46	—	9	1	7	—
87	27	37	—	14	10	18	—
100	20	45	--	6	12	10	—
Mean = 45.4 dots per seed				Mean = 10.4 dots per seed			
<i>t</i> = 0.7075. (60 D/F). Not significant.							

that the effect of increasing the dosage of the **Dt** gene is more nearly exponential than linear. More data will be obtained on this problem when the proper lines have been synthesized.

If the conclusions reached on the dosage effects of **a₁** and **Dt** are valid, it should be possible to predict the outcome of previously untried combinations. A plant of **a₁a₁^PDt dt** constitution was self-pollinated. The resulting seeds were classified into 102 pale coloured: 92 pale coloured with dots: 50 colourless with dots: 16 colourless. This is a close approximation to the expected ratio of 6:6:3:1. It was predicted before the dots were counted that the mean number per seed in class **a₁Dt**, colourless background with dots, would be twice that of the class **a₁^Pa₁Dt**, pale background with dots. The mean number of dots on the fifty seeds comprising the three **a₁Dt** classes was 45.0, while the mean number of dots on the ninety-two seeds of the six **a₁^Pa₁Dt** classes was 23.0.

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The dotted aleurone character is expressed only in seeds of a_1CRA_2Dt constitution. Dominant **C**, **R** and A_2 are, therefore, as necessary for the expression of the dotted character as recessive a_1 . The number of dots has been shown to be dependent upon the number of doses of a_1 . What the effect would be if the number of doses of dominant **C** or **R** or A_2 varied is a problem for the future as little information has been obtained. Certain data, however, suggest that seeds with one dominant **C** gene have fewer dots than seeds with two dominant **C** genes. This problem will, of course, be prosecuted, as it promises to open up an extensive field of investigation on the dosage effects of the several genes affecting aleurone colour.

CONCLUSION

1. A new dominant gene **Dt** interacts with recessive a_1 in the presence of the dominant alleles of the other factors concerned with aleurone colour to give coloured dots scattered over the aleurone layer. **Dt** is specific in its interaction with recessive a_1 .

2. The effect on the dotted character of varying the dosage of recessive a_1 was additive. Data on three dosages of a_1 were obtained.

3. The effect on the number of dots of varying the dosage of the **Dt** gene was non-additive.

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NOTE ON THE ORIGIN OF TRIPLOIDY IN MAIZE¹

By M. M. RHOADES²

TRIPLOID individuals arise sporadically in diploid populations of maize. It has been assumed that these triploids came from the fertilization of a diploid or unreduced egg with a haploid sperm because in other plants this has apparently been the usual manner of origin. Recently, however, triploidy in maize has occurred in another way. It would seem pertinent, therefore, to consider the different ways by which triploids have arisen.

CONTRIBUTION OF THE DIPLOID NUMBER OF CHROMOSOMES BY THE FEMALE PARENT

A failure of the chromosomes to disjoin normally in the anaphase of the first meiotic division may result in the formation of a restitution nucleus containing the unreduced number of chromosomes. Beadle (1930) found triploid individuals in the cross of asynaptic maize by haploid pollen. The diploid eggs arose through non-disjunction of the entire chromosomal complement brought about by the lack of pairing of the homologous chromosomes. Diploid eggs in normal strains of maize have arisen, presumably, through some disturbance in the meiotic processes, but no critical proof of this has been reported.

Non-disjunction of the haploid chromosome set in any of the three somatic mitoses of the female gametophyte could give rise to an egg possessing the unreduced number of chromosomes. One case of this kind has been reported in maize (Rhoades, 1933).

CONTRIBUTION OF THE DIPLOID NUMBER OF CHROMOSOMES BY THE MALE PARENT

The male parent could, theoretically, contribute the diploid number of chromosomes. The origin of triploidy in this manner, however, has not been previously reported.

In the summer of 1934 a triploid individual appeared in the writer's

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cultures in the progeny of a cross between two diploid genetic stocks. The female parent was homozygous for the gene glossy 1 (gl_1), while the male parent was homozygous for the white sheath 3 (ws_3) gene. Both of these factors are recessive. Glossy 1 is located in chromosome 7 and white sheath 3 in chromosome 2. Fortunately, this triploid plant was involved in several crosses with gl_1 and ws_3 plants, so a test of its genetic constitution was possible. In the cross of the triploid as the female parent by a male homozygous for gl_1 there were 89 non-glossy to 20 glossy plants in the F_1 progeny. This ratio is extremely close to that expected from the progeny of a trisomic individual carrying two dominant and one recessive allelomorphs and indicates that the constitution of the triploid, as far as the gl_1 locus was concerned, was $G1, G1, gl_1$. In the crosses with the triploid used as the pollen parent on silks of plants homozygous for the ws_3 gene the ensuing F_1 progeny consisted of 42 non-white sheath to 90 white sheath plants. These data suggest that the constitution of the triploid was the Ws_3, ws_3, ws_3 , since a 1 : 2 ratio is obtained from trisomic plants with one dominant and two recessive allelomorphs when used as pollen parents in a back-cross. As the triploid had only one recessive gl_1 gene and two ws_3 genes the conclusion that the diploid number of chromosomes was contributed by the male plant seems logical.

Contribution of the diploid number of chromosomes by the male parent could have been accomplished in one of three ways. The triploid individual could have arisen from the union of a haploid egg with a sperm from a diploid male gametophyte. The recent work of Randolph (1935) on reciprocal matings of diploid and tetraploid maize shows that diploid pollen usually is incompatible with diploid silks, although a viable embryo occasionally results. Sprague (1932) interpreted certain genetic data on hetero-fertilization as indicating that the egg and two polar nuclei of a single embryo-sac were fertilized by sperm from different male gametophytes. Sprague's assumption that more than one pollen tube could penetrate the embryo-sac was confirmed by the unpublished cytological observations of Virginia H. Rhoades who found that 11 per cent. of the embryo-sacs examined contained more than one pollen tube. In view of these facts there is the possibility that the triploid individual could have arisen from the simultaneous fusion of two haploid sperm nuclei with a haploid egg. There also is the possibility of non-disjunction of the haploid chromosome set of the generative nucleus during the formation of the male gametophyte. This would give a male gametophyte with a single diploid sperm nucleus and a haploid vegetative nucleus.

The compatibility of this type of male gametophyte on diploid silks is unknown. On this supposition the endosperm would need to be fertilized by a sperm from an additional pollen grain. The writer is inclined to believe the second explanation to be the more likely. Irrespective of the exact mechanism, however, the available data indicate that the diploid number of chromosomes was contributed by the male parent.

SUMMARY

Triploid individuals in maize occur through the fertilization of diploid or unreduced eggs by haploid sperm. The diploid eggs have arisen by complete non-disjunction of the entire chromosome set during meiosis or through non-disjunction during one of the three mitotic divisions of the female gametophyte. In either of these eventualities, however, the female gamete carried the diploid number of chromosomes.

Triploidy in maize is here shown to have arisen when the diploid number of chromosomes was contributed by the male parent.

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SOME EXPERIMENTS WITH *GERANIUM* SPECIES

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THE late W. C. F. Newton started the investigation of several interesting crosses between species of *Geranium* in 1920. Since 1927 Prof. L. Newton, J. F. S. Rudge, A. C. Fabergé and F. W. Sansome have attempted to carry on the work at the John Innes Horticultural Institution. Unfortunately the seed production under controlled conditions is meagre and the percentage of germination is low. For example, it has been usual to obtain 2-3 plants from the pollination of all the flowers on one plant during a period of 2 months. Having regard to these difficulties it has been decided to put the scanty but suggestive data on record and to discontinue the work.

HYBRIDS BETWEEN *G. ENDRESSI* GAY AND *G. STRIATUM* L.

Table I contains the results of the various crosses made between these two species.

TABLE I

Family	Cross	Normal plants	Male sterile plants
25/22	<i>Endressi</i> × <i>striatum</i> F_2	2	1
26/22	<i>striatum</i> × <i>Endressi</i> F_2	4	—
1/23	<i>Endressi</i> × <i>striatum</i> F_2	4	1
2/23	<i>striatum</i> × <i>Endressi</i> F_2	4	—
1/25	<i>striatum</i> × <i>Endressi</i> F_2	1	—
2/25	<i>Endressi</i> × <i>striatum</i> F_2	1	—
3/25	25/22 ♂-sterile × <i>striatum</i>	—	1
4/25	1 ¹ /23 ♂-sterile × <i>striatum</i>	—	1
5/25	1 ¹ /23 ♂-sterile × <i>Endressi</i>	7	—
6/25	1 ² /23 ♂-sterile × <i>striatum</i>	—	5
7/25	1 ² /23 ♂-sterile × <i>Endressi</i>	1	—
9/26	1 ¹ /23 ♂-sterile × <i>striatum</i>	—	2
10/26	1 ¹ /23 ♂-sterile × <i>Endressi</i>	1	—
11/26	1 ¹ /23 ♂-sterile × (<i>E.</i> × <i>s.</i>) F_1	1	1
12/26	1 ¹ /23 ♂-sterile × (<i>s.</i> × <i>E.</i>) F_1	5	3
2/27	1 ¹ /23 ♂-sterile × (<i>s.</i> × <i>E.</i>) F_1	3	1
3/27	1 ² /23 ♂-sterile × (<i>s.</i> × <i>E.</i>) F_1	2	—
1/28	1 ² /23 ♂-sterile × (<i>s.</i> × <i>E.</i>) F_1	10	8
2/28	1 ² /23 ♂-sterile × (<i>s.</i> × <i>E.</i>) F_1	10	18
3/28	1 ² /23 ♂-sterile × (<i>E.</i> × <i>s.</i>) F_1	1	4
4/28	(<i>E.</i> × <i>s.</i>) F_1 × (<i>s.</i> × <i>E.</i>) F_1	4	5
5/28	(<i>s.</i> × <i>E.</i>) F_1 × (<i>E.</i> × <i>s.</i>) F_1	5	—
6/28	(<i>E.</i> × <i>s.</i>) F_1 × <i>striatum</i>	3	2
7/28	<i>Endressi</i> × (<i>s.</i> × <i>E.</i>) F_2	22	7

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The facts suggest that a factor carried by *G. striatum* when homozygous in the cytoplasm derived from *G. Endressi* causes the contabescence and petaloidy of the anthers. The plants carrying flowers with petaloid anthers have distinctive characteristics. The petals are smaller in size, very narrow and deeper in colour than the normal *striatum*; the stamens are white in colour and the anthers more or less petal-like. The last characteristic is variable in degree, but empty and deformed pollen sacs are a constant feature. The leaves of the plant also may show differences from the hermaphrodite plant, under dry conditions the leaves of the male-sterile plants are narrower and more liable to shrivel. The evidence regarding segregation is summarized thus:

TABLE II

	Normal	Petalloid anthers
<i>Endressi</i> × <i>striatum</i> F_2	7	2
<i>striatum</i> × <i>Endressi</i> F_2	5	—
F_2 ♂-sterile × <i>striatum</i>	—	10
F_2 ♂-sterile × <i>Endressi</i>	8	—
F_2 ♂-sterile × F_1	32	35
(<i>E.</i> × <i>s.</i>) F_1 × (<i>s.</i> × <i>E.</i>) F_1	4	5
(<i>s.</i> × <i>E.</i>) F_1 × (<i>E.</i> × <i>s.</i>) F_1	5	—
(<i>E.</i> × <i>s.</i>) F_1 × <i>striatum</i>	3	2
<i>Endressi</i> × (<i>s.</i> × <i>E.</i>) F_2	22	7

The postulated factor carried by *G. striatum* is recessive to an allelomorph carried by *G. Endressi*.

The crosses were made primarily to test the foregoing hypothesis but some evidence regarding the inheritance of other characters was obtained.

The contrasting characters studied were:

	Pollen	Petal-veins	Stigma	Purple spot on leaf
<i>G. Endressi</i>	Blue	Dark	Red	Present
<i>G. striatum</i>	Yellow	Clear	Light yellow	Absent
F_1 's	Blue	Dark	Light yellow	Faint spot

The F_2 segregation was:

Dominants	Recessives	Dominants	Recessives
Blue	Yellow pollen	45	14
Dark	Light veins	52	12
Yellow stigma	Red	48	15
Purple spot	No spot	46	16

There is no sign of linkage between these characters. Other crosses were made but most of them provided little useful data concerning the segregation of these characters, since their parents were F_2 plants of unknown genotype and the families were of small size.

G. SANGUINEUM L., *G. SANGUINEUM* VAR. *ALBUM*, G.
LANCASTRIENSE MILL.

These three forms of *G. sanguineum* are found in the wild, and differ from one another in a number of characters. The following differences were studied in the experiments.

	Flower colour	Petal-veins	Pollen	Bud	Habit
<i>G. sanguineum</i> L.	Magenta	Dark	Blue	Revolute	Sub-erect
<i>G. sanguineum</i> var. <i>album</i>	White	Clear	Yellow	Imbricate	Erect
<i>G. lancastriense</i> Mill	Pale	Dark	Yellow	Imbricate	Prostrate

The data obtained are shown in Table III.

TABLE III

Cross	Flower colour Dominant- magenta	Recessive (white or <i>lancastriense</i>)
F_3 <i>lanc.</i> \times <i>sang.</i>	92	—
F_3 <i>lanc.</i> \times <i>album</i>	73	—
F_2 <i>album</i> \times <i>lanc.</i>	2	—
F_2 <i>sang.</i> \times <i>album</i>	11	—
F_1 (<i>lanc.</i> \times <i>sang.</i>) \times <i>sang.</i>	20	—
F_1 (<i>sang.</i> \times <i>album</i>) \times <i>album</i>	4	—
F_1 (<i>lanc.</i> \times <i>album</i>) \times <i>album</i>	13	2 white
F_2 (<i>lanc.</i> \times <i>album</i>) \times <i>lanc.</i>	40	1 <i>lanc.</i> , 2 mericlinal
F_2 (<i>sang.</i> \times <i>album</i>) \times <i>album</i>	13	2 white

Only one plant of each form or F_1 was used throughout the crosses in this table.

The F_1 and F_2 hybrids between any two of these forms were, with a few exceptions to be mentioned, all similar in flower colour, imbricate veneration of flower bud, blue pollen colour and distribution of colour in the veins. The habit varied from suberect to subprostrate in the F_1 and most of the F_2 plants, but the latter showed a greater range and occasionally approached the habits of *album* or *lancastriense*. Two plants in the F_2 of *lancastriense* \times *album* had paler magenta flowers, revolute petals and yellow pollen. One of these exceptional plants was used in breeding and gave, on crossing with *lancastriense*, one plant of *lancastriense* type, with prostrate habit, *lancastriense* petal colour, veins and pollen, and two plants which were mericlinal chimaeras for flower colour (*sanguineum*-magenta and *lancastriense*-pink (see Table III).

Table III summarizes the segregation of flower colour in these crosses. It will be seen that the recessive colours are rare in occurrence, although the total number of plants involved is rather small. The fact that recessives were only recovered after back-crossing F_2 plants which were vaguely different from their sister plants is interesting. Early in the

investigation it was thought that *lancastricense* and *album* contributed complementary factors for *sanguineum* flower colour and this appears to be confirmed by the available data. The scarcity of recessives was only accounted for when it was discovered that the chromosome number of these forms was $2n=84$, whereas other *Geranium* species have $2n=28$. The chromosome number was ascertained by Mr Rudge. Dr Warburg, Botany Department, Cambridge University, who has made a special study of the cytology of the Geraniaceae, has confirmed this chromosome number in a private communication. It is therefore suggested that auto-hexaploid ratios are here involved. Using Haldane's formula for calculating the gametic output, we find that the segregation from hexaplex dominant \times recessive would be 399 dominant : 1 recessive; duplex \times recessive nulliplex would be 4 dominant : 1 recessive; simplex \times recessive nulliplex would be 1 dominant : 1 recessive (chromosomal or complete reductional segregation).

On this basis the F_2 plants backcrossed to the recessive parent may be triplex and duplex respectively, the 42 : 1 and 13 : 2 ratios corresponding to the theoretical 40.8 : 2.2 and 12 : 3 ratios. Among the 178 plants of the F_2 we should expect the following plants:

	A_6	A_5	A_4	A_3	A_2	A_1	a
Approx.	0.44	8.1	44	72.16	44	8.1	0.44

if the segregation of the genes followed strictly chromosome segregation in an auto-hexaploid unaccompanied by other disturbing influences. It will therefore be seen that such theoretical considerations might account for the reduced segregation of flower colour in the F_2 . The occurrence of two plants, some of the flowers of which were mericlinal chimaeras for *sanguineum* and *lancastricense* may be significant. These occurred in one family where (on this theory) simplex plants were to be expected most frequently and they may indeed be simplex forms. An attempt was made to test these plants and other parts of the general problem by critical crosses but the practical difficulties mentioned earlier were too great.

The remaining characters behaved in a similar manner to that of flower colour; the important point, however, is that plants recessive for flower colour were recessive for the other characters, and the particular plants used in the F_2 generations for crossing were selected because they carried recessive, or at least, different characteristics from the remaining F_2 plants. Hence it is possible that there is close linkage between these characters. Naturally it was not possible to obtain definite information with this refractory material.

SUMMARY

From the reasons stated, although there is no proof, the evidence indicates that:

(1) There is a factor carried by *G. striatum* which, in the presence of cytoplasm of *G. Endressi*, causes petaloidy of the anthers.

(2) There is autohexaploid segregation of flower colour in *G. sanguineum*.

REFERENCE

- HALDANE, J. B. S. (1930). "Theoretical genetics of autopolyploids." *J. Genet.* **22**, 359-72.

THE PHYSIOLOGICAL CONSEQUENCES OF POLYPLOIDY¹

I. GROWTH AND SIZE IN THE TOMATO

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(With Two Text-figures)

INTRODUCTION

EVER since the discovery of polyploidy, descriptions of the properties which distinguish one member of a series from another member have been published. Polyploidy, as a variation, is quite general throughout the vegetable kingdom, and it is clearly of great interest to derive some generalization which would enable one to predict the effect of chromosome doubling in any given case. As a result of modern cytogenetic work, such predictions can be made with great certainty as regards the genetical and cytological consequences, and the effect on self- and cross-fertility. The physiological results of polyploidy, on the other hand, are only known very superficially. The present paper is an account of an investigation of the effect of polyploidy on growth and total size in the tomato. The same data will be re-examined from the point of view of variability in a separate paper.

The tomato is one of the few plants in which it is possible to induce tetraploidy at will, and therefore to obtain rigorously comparable material (Jørgensen, 1928). From the moment the chromosomes of an organism are doubled, it begins to exist under what may be termed a new genetic régime. It is difficult to estimate how important the effects of the early stages of this process are as compared with the effects of polyploidy as such. Therefore, in the present investigation, no material was used which had existed as a tetraploid for more than three generations. All critical conclusions were derived from material in the first generation after somatic doubling.

¹ In part adapted from a thesis accepted for the degree of Ph.D. of the University of London.

REVIEW OF LITERATURE

For the reasons stated above, it is difficult to obtain valid information from polyploids found in nature. Müntzing (1936) has given a very full review of literature on autopolyploids in general. Only polyploids produced experimentally will be considered here.

The work on mosses has been reviewed by Schwarzenbach (1926), v. Wettstein (1932) and Allen (1935). Cell volume is usually roughly proportional to chromosome number, but there are exceptions to this, and in the case of *Anthoceros laevis* the diploid has smaller cells than the haploid (Schwarzenbach, 1926). Becker (1932) has found that osmotic pressure decreases as cell size increases. The situation in ferns has been reviewed by Andersson-Kottö (1936); cell sizes behave in the same way as in the mosses.

In the tomato, differences in appearance and growth have frequently been reported (e.g. Sansome, 1933), but usually no actual measurements were taken. There is no doubt that tetraploids can be recognized from diploids with a good deal of certainty, but the difference is hard to define. According to Sansome & Zilva (1933, 1936) the vitamin C content of tetraploid fruits appears to be greater, though this has been questioned by MacHenry and Graham (1935). Kostoff & Aksamitnaja (1935) have published chemical analyses of diploid and tetraploid petunia and tomato, but used only two replicates, so that it is difficult to base any conclusions on their work.

EXPERIMENTAL RESULTS

The numerical data accumulated in this work are bulky, and every effort has been made to summarise them as briefly as possible. Consequently, tests of significance alone are most often given. Fisher's (1935, 1936) statistical notation is used throughout. In the experiments about to be described all populations which have been inbred under controlled conditions for a few generations are called pure lines, for the sake of convenience.

1934 GROWTH-RATE EXPERIMENT

This was a preliminary experiment, principally designed to show whether tetraploids differ consistently from diploids in their growth. Information was also desired, in order to establish a technique for future experiments, on the relation between the dry and fresh weight throughout the period considered, and on the relation between roots and shoots.

Description of material. Four pure lines, *P*, *Q*, *R* and *S*, were used, which had been inbred for five generations. These may be classified in a fourfold table:

Chromosome number

2x 4x

<i>P</i>	<i>R</i>	... + ... d p o s r y } Genetic constitution
<i>Q</i>	<i>S</i>	

where **d**=dwarf, **p**=peach (hairiness), **o**=oval fruit, **s**=compound inflorescence, **r**=red colour of fruit, **y**=colourless skin of fruit. Full descriptions of these factors are given by MacArthur (1931). The two tetraploid lines were doubled in 1932.

Methods and technique. The plants were grown in water culture ($\frac{1}{2}$ strength Knop) in paraffined earthenware pots 23 cm. across and 26 cm. deep, each holding 5.25 litres. The solution was renewed each week; aerating was found unnecessary for tomatoes. The lids, of compressed cork 8 mm. thick, had twenty 2.5 cm. equally spaced holes bored in them. The lids were soaked in paraffin, and a sheet of muslin placed on one side while the wax was still melted; small seedlings could then be pushed through a small hole in the muslin. When samples were taken, the whole 2.5 cm. disc of muslin was cut out with the plants. Seeds were germinated on moist filter paper in Petri dishes. The pots were kept on the staging of a greenhouse, the temperature of which was kept at about 24° C.

The experiment was laid out in eight randomized blocks, four pots of twenty plants each per block. Samples were taken every 4 days, and always at the same time of the day; nine samples were taken altogether. Within each pot the plants were taken in a predetermined systematic way in order to ensure their gradual spacing and to avoid bias. Each plant was cut in two at cotyledon level and the fresh weight of both "roots" and "shoots" recorded separately. Plants were then dried in flat aluminium dishes in a water oven ($\pm 98^\circ$ C.) for 20 hours; they were then weighed again. All the weighings were done on a battery of Joly balances of phosphor bronze wire (Bolas & Melville, 1933) so that each weighing is accurate to at least 0.01 of its value. Weighing is very rapid and consequently no precautions need be taken against the absorption of moisture.

Thus four measurements were taken on each individual. It was

originally intended to take only one plant from each pot at each time. Actually twelve plants of each line were taken on each occasion instead of eight. Of these twelve, eight were taken one from each block, and four from each of four blocks, the particular groups of four blocks being a different one on successive occasions. This results in a partial confounding of "times", and of 32 of the 88 degrees of freedom for the "lines" \times "times" interaction with one of the 7 degrees of freedom for blocks on each occasion. The total contribution for blocks is quite insignificant, as the following table shows; dry weights of the shoots of eight individuals sampled, one from each block, are used:

TABLE I

Variance	D.F.	S. of S.	M.S.	z	0.05 point of z
Blocks orthogonal to times	7	0.2588	0.0370	0.2172	0.3565
Error (from replication within blocks)	394	9.4187	0.0239		

¹ In this, and in all following tables D.F. = degrees of freedom, S. of S. = sum of squares, M.S. = mean square, 0.05 point of z = that value in the distribution of z the probability of obtaining which by random sampling is 1 in 20, from Fisher's table (Fisher, 1936).

On the strength of this information, the sum of squares for blocks is not subtracted from error in any of the following analyses, and all twelve measurements in each line on each occasion are included.

All the computations are made on logarithms to base 10 of the weight, 0.1 mg. being the unit. The result of using a logarithmic scale is that increase of weight with time is very nearly linear, depending on the constancy of the environment. The plants were in the so-called "grand" period, and grew exponentially, as it is expressed by Blackman's equation:

$$W_t = W_0 e^{rt},$$

where W_0 is the initial weight, W_t the weight at time t , and r the relative growth rate or efficiency index (Blackman, 1919). In the analysis of variance, the sum of squares for times is determined by all the differences between samples taken at different times. These differences will be largely a function of the relative growth rate, but will also arise from changes of environment with time. The relative importance of these two may be examined by separating the sum of squares for times into two portions: one accounted for by a linear regression and a remainder. This is done in Table II. It will be seen that although by far the greater part is accounted for by that one degree of freedom for the linear regression, the remainder, based on 7 degrees of freedom, is still highly significant. This means that the relative growth rate was not constant.

The times \times lines interaction is made up of differences in the effect of time on the different populations. This will consist of differences in relative growth rate and of differences in response to changes of environment. It is not unreasonable to suppose, *a priori*, that differences of the effect of environment on different strains will be small in comparison with the effect of changes of environment as a whole. That this is in fact so can be seen from Tables VIII and IX, in which a component due to a linear regression is separated from the sums of squares corresponding to various comparisons. In two cases the remainders are not significant, and in two others they are not very large.

TABLE II

Decomposition of times

Variance	D.F.	S. of S.	M.S.	z	$0.01 z$
Times	8	296.6730	37.0841	—	—
Linear regression	1	292.3482	292.3482	—	—
Remainder	7	4.3482	0.6212	1.6289	0.4963
Error	394	9.4187	0.0239	—	—

TABLE III

Dry weight of shoots: main analysis

Variance	D.F.	S. of S.	M.S.	z	$0.01 z$
Total	429	309.2647	0.7209	—	—
Times	8	296.6730	37.0841	—	—
Lines	3	1.7827	0.5941	1.6067	0.6717
T. \times L.	24	1.3903	0.0579	0.4424	0.3014
Error	394	9.4187	0.0239	—	—

Such a procedure results in a gain of precision, to the extent to which changes in environment will tend to affect different lines to the same extent. It is the existence of this method which made it possible deliberately to change the environment of a whole experiment by transferring the plants from a greenhouse to a patch of land out of doors in 1935.

The criterion of classification "lines" gives a means of testing differences in the total amount of weight produced. Biologically it is a function of both the initial weight W_0 and the growth rate r of the compound interest law.

The main analysis of variance is given in Table III. As will be seen later, the correlation between the four measurements taken on one plant is so high that one of them may safely be taken as representative. The dry weight of shoots alone is used.

Two plants were accidentally lost in sample 3 of line *P*, hence the total number of degrees of freedom is not 431, but 429. Owing to the trivial difference, the samples are treated as if they were orthogonal, for the sake of simplicity.

A decomposition of the three degrees of freedom for lines is given in Table IV. Fisher's factorial notation is used (Fisher, 1935). The letters *p*, *q*, *r*, *s* represent the four lines. The comparisons:

$$p + q - r - s,$$

$$p - q + r - s,$$

$$p - q - r + s,$$

are orthogonal to one another. The first represents the effect of polyploidy, and its mean square is actually less than that for error, so that the tetraploids did not differ significantly from diploids in the total amount of matter produced. The second comparison represents the effect

TABLE IV

Dry weight of shoots: decomposition of "lines"

Variance	D.F.	S. of S.	M.S.	<i>z</i>	0.01 <i>z</i>
Lines (total)	3	1.7827	0.5942	1.6067	0.6717
$p + q - r - s$	1	0.0005	0.0005	—	0.9511
$p - q + r - s$	1	0.8469	0.8469	1.7838	
$p - q - r + s$	1	0.9350	0.9350	1.8333	
$p + q + r - 3s$	1	1.2089	1.2084	1.9616	
Remainder	2	0.5743	0.2872	1.2431	0.7695
Error	394	9.4187	0.0239	—	—

of the factor *d* (which is the only one of the six factors that might be expected to affect growth appreciably), and is significant. The last comparison represents the extent to which this fourfold classification fails to account for the differences, and it actually gives the biggest mean square of the three.

The comparison $p + q + r - 3s$ is not orthogonal to the first three; it is suggested by the graph on Fig. 1, from which it appears that *s* is smaller than the other three lines. This gives a highly significant mean square.

The composition of the times \times lines interaction is similarly analysed on Table V. For each comparison, a component accounted for by a linear regression is isolated.

It will be seen that this component is large in the first two comparisons of Table V. $t(p - q + r - s)$ is not itself significant, but it appears quite legitimate to isolate the linear component since there are good *a priori* reasons for so doing. The third comparison, which, as was already seen, tests the failure of the fourfold classification, is significant,

but its linear component is quite small and not significant at all. Thus, polyploidy and the factor *d* account for differences in growth rate, but interact with one another in producing different deviations from a constant growth rate.

The comparison $t(p+q+r-3s)$ is very large, and the remainder from a linear regression is significant, though it fails to reach the 0.01 level of

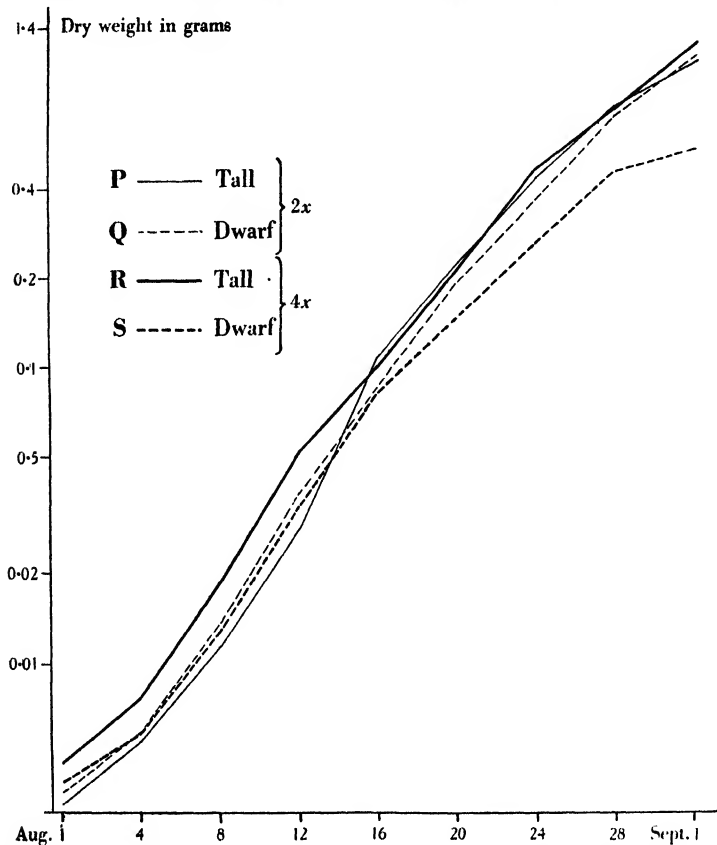


Fig. 1. The growth of the four lines *P*, *Q*, *R* and *S* represented on a logarithmic scale.

probability. The situation is clear on the graph in Fig. 1, where it will be seen that the line *s* grew at a slower rate during the second half of the experiment.

From these analyses the conclusion may be drawn that tetraploids do not produce significantly more material than diploids in the period considered, and that though differences in growth do exist, they are small and represent complex interactions with the genotype and the environment.

TABLE V

Dry weight of shoots: decomposition of "times \times lines" interaction

Variance	D.F.	s. of s.	M.S.	z	Points of z	
<i>TL</i> interaction (total)	24	1.3903	0.0579	0.4424	0.01	0.3014
<i>t</i> ($p+q-r-s$)	8	0.7983	0.0998	0.7147	0.01	0.4693
Linear regression	1	0.5593	0.5593	1.5764	0.01	0.9511
Remainder	7	0.2391	0.0342	0.1792	0.05	0.3565
<i>t</i> ($p-q+r+s$)	8	0.3048	0.0381	0.2332	0.05	0.3369
Linear regression	1	0.2616	0.2616	1.1965	0.01	0.9511
Remainder	7	0.0432	0.0062	—	—	—
<i>t</i> ($p-q-r+s$)	8	0.4935	0.0617	0.4742	0.01	0.4693
Linear regression	1	0.0553	0.0553	0.4194	0.05	0.6760
Remainder	7	0.4382	0.0626	0.4814	0.01	0.4963
					0.05	0.3565
<i>t</i> ($p+q+r-3s$)	8	0.8315	0.1039	0.7348	0.01	0.4693
Linear regression	1	0.4076	0.4076	1.4182	0.01	0.9511
Remainder from L.R.	7	0.4238	0.0605	0.4644	0.01	0.4963
					0.05	0.3565
Remainder	16	0.5589	0.0349	0.1893	0.05	0.2523
Error	394	9.4187	0.0239	—	—	—

Covariance of different measurements on the same plant

Similar analyses to the above were made on three other measurements, and gave almost identical results. Of the six possible covariances, only two were calculated: fresh \times dry weight of shoots and dry shoots \times dry roots. These are given in Tables VI and VII. Regression and correlation coefficients corresponding to each item in the analyses of variance are given, logarithms being used as before. Regression coefficients are there-

TABLE VI

Covariance of fresh and dry weight (shoots)

Source of covariance	D.F.	$S(x-\bar{x})(y-\bar{y})$	Correlation coefficient	Regression of F.W. on D.W.
Total	429	299.5511	0.989	0.969
Times	8	288.5222	0.999	0.972
Lines	3	1.7902	0.893	1.004
T. \times L.	24	1.4505	0.877	1.043
Remainder	394	7.7882	0.672	0.827

TABLE VII

Covariance of roots and shoots (dry)

Source of covariance	D.F.	$S(x-\bar{x})(y-\bar{y})$	Correlation coefficient	Regression of R. on S.
Total	429	286.5350	0.995	0.927
Times	8	276.3842	0.999	0.931
Lines	3	1.2545	0.913	0.704
T. \times L.	24	1.1690	0.857	0.841
Remainder	394	7.7273	0.888	0.820

fore independent of the units in which the two variables are expressed, and tend to unity as correlation increases. It will be seen that the correlations are in all cases very high.

The fact that the four lines do not differ significantly among themselves in water content (92.09 per cent) can be shown more simply by taking for each line the difference between the fresh and dry weight, and calculating a sum of squares for these differences. Since the two measurements are correlated, the variance with which this has to be compared is $V_{(a)} + V_{(b)} - 2W_{(ab)}$, where $V_{(a)}$ is the variance of the first variable, $V_{(b)}$ that of the second, and $W_{(ab)}$ their covariance. Table VIII shows that the mean square for differences is actually less than the corresponding error.

TABLE VIII

Lines	<i>P</i>	<i>Q</i>	<i>R</i>	<i>S</i>
Mean fresh weight <i>a</i>	3.9731	3.9667	4.0981	3.9115
Mean dry weight <i>b</i>	2.8723	2.8614	2.9475	2.7666
Difference Δ	1.1008	1.1053	1.1506	1.1449

$S(\Delta - \bar{\Delta})^2 = 0.002024$; m.s. = 0.00068; $V_{(a)} + V_{(b)} - 2W_{(ab)} = 0.01066$.

The relationship between roots and shoots in the four lines may be treated in the same way (Table IX). This again shows a mean square for differences which is less than error, so that shoots may safely be taken as representative of the whole plant. Roots represent 19.10 per cent of the total dry weight.

TABLE IX

Lines	<i>P</i>	<i>Q</i>	<i>R</i>	<i>S</i>
Mean dry shoots <i>a</i>	2.8723	2.8614	2.9475	2.7666
Mean dry roots <i>b</i>	2.2854	2.2939	2.3898	2.2572
Difference Δ	0.5869	0.5675	0.5577	0.5094

$S(\Delta - \bar{\Delta})^2 = 0.00326$; m.s. = 0.00109; $V_{(a)} + V_{(b)} - 2W_{(ab)} = 0.00500$.

Embryo weights of the lines, P, Q, R and S

In the first sample of the 1934 experiment, the plants weighed about 5.5 mg., which is about $5 \times$ the weight of the embryo in the ripe seed. It will also be seen on the graph of Fig. 1 that in the first sample the two tetraploids were heavier than the two diploids, though this is not statistically significant. It appeared of interest to determine the weights of the actual embryos.

Fifty embryos, together with the corresponding seeds, were weighed in each of the four lines. Seeds were first softened (but not germinated) by soaking overnight in 0.1 per cent. HgCl_2 . The embryos were then dissected out, examined under a binocular microscope, and dried. Thet Su & Ashby

(1929) have described a method for drying very small biological material, and the procedure followed here is partly adapted from theirs. Drying took place at 50° C. under about 5 mm. Hg pressure over P_2O_5 . Constant weight was attained in about 10 min., but the embryos, in batches of ten at a time, were left in the drier for half an hour. For weighing, a specially constructed torsion balance was used, which gave a precision by direct reading of 0.01 mg. (3 mm. on the scale) (the author, unpublished).

Seed and embryo weight are highly correlated, as the following table shows:

TABLE X

Covariance	$S(x - \bar{x})(y - \bar{y})$	Correlation coefficient	t
Total	334838	—	—
Between lines	139197	0.8524	23.268
Within lines	195641	—	—

The means of the four lines are given below, in mg.:

TABLE XI

Lines ...	$P(2x)$	$Q(2x)$	$R(4x)$	$S(4x)$
Mean seed weight	2.4446	2.6730	3.4902	3.1812
Mean embryo weight	0.9744	0.9972	1.3812	1.2470

Only the comparison $p+q-r-s$ need be considered; it is given on Table XII for embryo weights:

TABLE XII

Embryo weight

Variance	D.F.	s. of s.	M.S.	z	0.01 z
Total	199	152791	768.39	—	—
Lines	3	58523	19507.67	1.8514	0.6518
$p+q-r-s$	1	53890	53890.00	2.3595	0.9882
Remainder	2	4633	2316.28	0.7859	0.7755
Error	196	94268	480.96	—	—

Thus it appears that both tetraploid seed and tetraploid embryos are about 1.3 times heavier than the corresponding diploids, and these differences are highly significant. This advantage of increased embryo weight was lost during the eleven days which elapsed between seed sowing and the first sample of the growth-rate experiment. It is possible that the small difference which exists in the first sample is the last trace of the difference found in embryo weight.

1935 GROWTH-RATE EXPERIMENT

This experiment was mainly designed for the measurement of variability (to be dealt with elsewhere), but it is capable of yielding informa-

tion on growth in the same way as the 1934 experiment. In addition, comparisons can be made on heterosis in the F_2 population.

Description of material. Ten genetic populations of tomatoes were used:

- A* 2x Pure line, inbred for six generations. Genetic constitution: **dpos r y**.
- B* 4x Pure line inbred for six generations. Doubled in 1932, and taken from same stock as *A* in that year.
- C* 4x Pure line inbred for six generations. Genetic constitution **dpos r**.
- D* 4x Pure line inbred for three generations, genetic constitution: +.
- E* 4x } F_2 of **dpos r y** × **cluh**.
- F* 2x }
- G* 2x } F_2 of "Chinaman" × **dpos r y**.
- H* 4x }
- I* 2x Pure line "Chinaman".
- J* 2x Pure line inbred for at least six generations. Genetic constitution: **cluh**.

c=compound inflorescence, **l**=lutescent, **u**=uniform, **h**=hairy (MacArthur, 1931). "Chinaman" is a tomato variety originally obtained from Messrs Vilmorin-Andrieux and Cie of Paris. It has been cultivated since about 1860 by Chinese market gardeners in Australia, and is homozygous for several recessives, including *fasciated* and *brachytic*. Tetraploid F_2 's were obtained by somatic doubling of the F_1 and selfing. In the case of *G* and *H*, the same F_1 individual is the parent of both; *A* and *B* come from selfing the same individual in 1932.

Description of experiment. The experiment was arranged in four randomised blocks. Seeds were all sown on the same day (13 June 1935), three seeds per pot; 172 pots of each line were sown, forty-three pots per plot. The experiment was started in a greenhouse, and on 11 and 12 July all plants were transferred out of doors, two blocks being transferred on each of these two dates, and the position of lines within blocks was randomized again.

The first sample was taken on 26 July, and subsequently at intervals of one week; samples were always taken at the beginning of the afternoon. On each occasion 12 plants were taken from each of the ten lines, three from each block, thus making 120 plants per sample. The first two samples were taken before thinning out, the choice being random with the restriction that 1 plant only was taken from each pot. In the third

sample, three pots were taken, leaving forty pots per plot. Out-of-doors, the distance between plants was at first 40 cm., but on 6 August every other plant was thinned out, leaving a pattern of diagonal squares. The choice of the individuals to be taken at each out-of-door sample was pre-determined systematically, in order to ensure adequate spacing.

The plants were cut off at cotyledon level and dried in an oven built for the purpose. The temperature was first raised to 100° without air current to ensure rapid killing. After half an hour a fan was switched on which drove a stream of hot air over the plants, the temperature being maintained at 70° C. by means of a thermoregulator. Constant weight was attained in a few hours, depending on the size of the samples, but all samples were kept at 70° C. overnight. Plants were weighed on phosphor bronze Joly balances as was done in the 1934 experiment.

Eight samples at weekly intervals were taken. Thus there are 3 individuals \times 4 blocks \times 10 lines \times 8 times = 960 measurements. In such

TABLE XIII

1935 experiment: main analysis

Variance	D.F.	s. of s.	M.S.	<i>z</i>	0.01 <i>z</i>
Total	959	1038.131	1.0825	—	—
Times	7	963.324	137.6177	—	—
Lines	9	15.823	1.7582	1.8417	0.4199
<i>T. \times L.</i>	63	11.339	0.1800	0.7018	0.2134
Absolute error	640	28.289	0.0442	—	—
Residual error	240	19.356	0.0806	—	—

an experiment there are two kinds of experimental error. One represents differences between individuals classified alike with respect to all other criteria, and will here be called "absolute" and used for tests of significance. The other error is the one commonly used in the standard type of agricultural experiment, and consists of block interactions; it represents discrepancies of behaviour of groups classified alike but in different blocks. This will be called residual error.

Putting *L*=lines, *T*=times, *I*=individuals, *B*=blocks, the absolute error will contain, *I*, *LI*, *TI*, *IB*, *LTI*, *LIB*, *TIB* and *LTIB*, and will be based on 640 degrees of freedom. The residual error will contain *B* and all block interactions not containing *I*, i.e. *LB*, *TB* and *LTB*, and will therefore be based on 240 degrees of freedom. The two degrees of freedom for individuals cannot of course be separated.

The main analysis is given in Table XIII. Logarithms to the base 10 are again used; 1 mg. is a unit.

Every item in this general analysis is highly significant. In Table XIV

is given a decomposition of the residual error, which shows that the use of blocks did in fact increase the precision of this experiment.

TABLE XIV

Decomposition of residual error

Variance	D.F.	S. of S.	M.S.	<i>z</i>	0.01 <i>z</i>
Residual error	240	19.3557	0.0806	—	—
Blocks	3	3.3387	1.1129	2.7643	0.6655
Block interactions (excluding I)	237	16.0170	0.0676	0.1844	0.1285
Absolute error	640	28.2891	0.0442	—	—

No doubt this is due to the greater heterogeneity of a plot of land as compared with water cultures in a greenhouse. If all blocks and their interactions had been included in error as had been done for the 1934 experiment, the mean square for error would have been 0.05414 instead of 0.04420, the former being based on 880 degrees of freedom. The portion of the sum of squares for times which is taken up by a linear regression is shown in Table XV; it will be seen that the remainder is very significant.

TABLE XV

Decomposition of times

Variance	D.F.	S. of S.	M.S.	<i>z</i>	0.01 <i>z</i>
Times	7	963.3242	137.6177	—	—
Linear regression	1	921.5746	921.5746	—	—
Remainder	6	41.7496	6.9583	3.6808	0.5202
Error	640	28.2891	0.0442	—	—

The growth rates of all ten lines are shown in Fig. 2. The growth rate was slower in the field than in the greenhouse; no doubt this accounts for a large part of the remainder from the linear regression.

Of the 9 degrees of freedom for lines, three give relevant information when isolated. Using Fisher's factorial notation as before, they may be represented as:

$$\begin{array}{ll}
 (2x \longleftrightarrow 4x) & a - b - c - d - e + f + g - h + i + j \\
 (P.L. \longleftrightarrow F_2) & 2a + 2b + 2c + 2d - 3e - 3f - 3g - 3h + 2i + 2j \\
 (2x \longleftrightarrow 4x) (P.L. \longleftrightarrow F_2) & 2a - 2b - 2c - 2d + 3e - 3f - 3g + 3h + 2i + 2j
 \end{array}$$

They are independent of one another and so may be combined into one analysis. The first compares all diploids with all tetraploids. The second compares pure lines with F_2 populations. The third may be called the interaction of the first two degrees of freedom, and it will be seen that it is obtained by cross-multiplication of the coefficients of the first two functions.

The decomposition of the sum of squares for lines is given in Table XVI. The diploid-tetraploid comparison gives a mean square which is less than error. F_2 's, on the other hand, produced significantly more dry matter than pure lines. The third comparison is also less than the error, thus showing that heterosis does not differ significantly in diploids and tetraploids. The remainder is still very large, showing that causes other than those included in the above classification introduce big differences. This result entirely agrees with that obtained in 1934, namely that tetra-

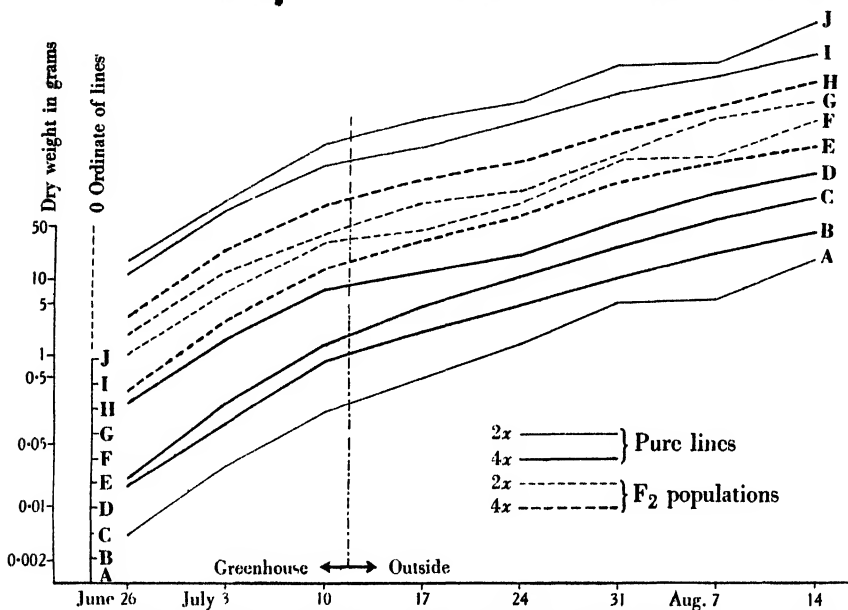


Fig. 2. The growth of the ten lines A, B, C, D, E, F, G, H, I and J represented on a logarithmic scale. The origins of the scale for each line are staggered, so as to space the curves.

ploids do not produce significantly more matter than diploids, and extends this conclusion to a period of two months after sowing.

The decomposition of the times \times lines interaction is given in Table XVII. Three groups of seven degrees of freedom each, which correspond to the three degrees of freedom of the "lines" analysis, are isolated.

The diploid-tetraploid comparison gives a significant mean square, but that component in it which is due to a linear regression is not significant. It is thus not due to a general difference in growth rate, but to different changes of the growth rate throughout the period. The remainder from the linear regression only just fails to reach the 0.01 level of significance.

Both the variance due to the pure line— F_2 comparison and its linear component are significant, and the remainder, while not very large, is still above the 0.05 level of significance. But the F_2 's grew *more slowly* than pure lines, so that the increase in the amount of dry matter produced, which was shown in the "lines" analysis, took place *in spite* of a slightly slower growth rate. It must thus be due to a greater initial weight. In this respect the data are in entire agreement with those of Ashby on maize (Ashby, 1930, 1932). Dr Ashby has kindly shown me some of his

TABLE XVI
Decomposition of lines

Variance	D.F.	S. of S.	M.S.	z	0.01 z
Lines (total)	9	15.8235	1.7582	1.8417	0.4199
($2x \longleftrightarrow 4x$)	1	0.0024	0.0024	—	—
(P.L. $\longleftrightarrow F_2$)	1	1.8799	1.8799	1.8749	0.9492
($2x \longleftrightarrow 4x$) (P.L. $\longleftrightarrow F_2$)	1	0.0249	0.0249	—	—
Remainder	6	13.9163	2.3194	1.9802	0.5202
Error	640	28.2891	0.0442	—	—

TABLE XVII
Decomposition of TL interaction

Variance	D.F.	S. of S.	M.S.	z	Points of z	
T.L. interaction	63	11.3389	0.1800	0.7018	0.01	0.2134
t ($2x \longleftrightarrow 4x$)	7	0.8907	0.1272	0.5286	0.01	0.4931
Linear regression	1	0.1426	0.1426	0.5856	0.05	0.6748
Remainder	6	0.7480	0.1247	0.5184	{ 0.01	0.5202
					{ 0.05	0.3740
t (P.L. $\longleftrightarrow F_2$)	7	1.0347	0.1478	0.6036	0.01	0.4931
Linear regression	1	0.4522	0.4522	1.1627	0.01	0.9492
Remainder	6	0.5828	0.0971	0.3934	{ 0.01	0.5202
					{ 0.05	0.3740
t ($2x \longleftrightarrow 4x$) (P.L. $\longleftrightarrow F_2$)	7	0.2226	0.0318	—	—	—
Linear regression	1	0.0563	0.0563	0.1209	0.05	0.6748
Remainder	6	0.1663	0.0277	—	—	—
Remainder	42	9.1908	0.2188	0.7998	0.01	0.2345
Error	640	28.2891	0.0442	—	—	—

new and unpublished data, which show that heterosis in tomatoes is of the same nature as in maize.

None of the items of the interaction of the $2x \longleftrightarrow 4x$ and P.L. $\longleftrightarrow F_2$ comparisons are significant, showing that the differences in growth of pure lines and F_2 populations are the same in both diploids and tetraploids.

The remainder from all three comparisons, based on 42 degrees of freedom, is still very significant, so that other factors introduce big differences.

DISCUSSION

From these experiments it appears that the difference in size between diploid and tetraploid tomatoes which has been reported on several occasions is a deceptive appearance. It should be noted that in no case where this difference was described have any actual measurements been given.

In the case of the 1934 experiment, it was seen that the initial advantage of 30 per cent heavier embryos was lost during the 11 days following sowing. It is conceivable that in another environment this would not be so, and that the adult plant would benefit from all or part of this initial advantage. If more definite information is to be gained on this matter, differences in external conditions must be introduced into the experiments as well as the diploid-tetraploid difference. It is a well-known principle in agricultural experimentation that a better variety may only be superior in being able to profit from a better environment. In cereal variety trials differences in sowing distance are often introduced because a higher-yielding variety will only get a chance if given more space. A similar principle may well apply to polyploidy. In this connection it is of interest to note that the geographic distribution of polyploid races is often strikingly different—a subject the data on which have recently been collected together by Müntzing (1936).

In the experiments reported here, the effect of polyploidy on the growth rate showed complex interactions with the genotype. In any case, these effects are relatively small and can only be detected by the special technique used. But the danger of drawing conclusions from experiments with only one genotype is evident.

SUMMARY

1. Experiments are described which are designed to compare diploid and tetraploid tomatoes from the standpoint of growth and total size. The data are treated by analysis of variance.

2. It is shown that tetraploids do not differ consistently from diploids in the total amount of substance produced. Polyploidy interacts in a complex way with the genotype to produce small deviations from a constant growth rate.

3. Tetraploid embryos are about 30 per cent heavier than the diploid, but this advantage is lost during germination.

4. Tetraploids do not differ significantly from diploids in their water content.

5. Heterosis in the F_2 is only due to increased initial weight, and it is of the same magnitude in diploids and tetraploids.

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THE PHYSIOLOGICAL CONSEQUENCES OF POLYPLOIDY¹

II. THE EFFECT OF POLYPLOIDY ON VARIABILITY IN THE TOMATO

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INTRODUCTION

THE data of the experiments described in an earlier paper (Fabergé, 1936) in which the effect of polyploidy on growth and total size was considered, lend themselves very well to an analysis of variability. One of these experiments, called the 1935 growth-rate experiment, was in fact mainly designed for the purpose. Full experimental details are given in that paper, and will not be repeated here.

Lindstrom (1935) studied the frequency distribution of fruit weight in diploid and tetraploid F_2 populations of *Solanum racemigerum* \times Tomato. According to Lindstrom, the tetraploid condition results in:

- (a) a reduction of fruit weight;
- (b) a reduction in relative variability;
- (c) the disappearance of positive skewness on an arithmetic scale.

When the data are transposed into a logarithmic metric, the positive skewness of the diploid vanishes, but the tetraploid becomes negatively skew. The reduction in relative variability is attributed to tetrasomic segregation ratios, and a general dominance of quantitative factors—in this case for small size. In this way a lesser proportion of recessive phenotypes appear in a tetraploid F_2 as compared with a diploid, and a narrower frequency distribution results. Lindstrom interprets his skewness result by assuming that gene action is in this case mainly additive, and not geometric, in the sense of Rasmusson (1933). Fisher's (1935, 1936) methods of analysis of variance have been used in analysing the present data, and his terminology is used throughout.

¹ In part adapted from a thesis accepted for the degree of Ph.D. of the University of London.

EXPERIMENTAL DATA

1934 experiment

In the 1934 experiment four inbred populations (six generations of selfing) *P*, *Q*, *R* and *S* were used. These may be classified as follows:

Chromosome number			
2x	4x		
<i>P</i>	<i>R</i>	+ diposry	} Genetic constitution
<i>Q</i>	<i>S</i>		

The two tetraploids had been obtained by somatic doubling in 1932, and the corresponding diploids came from selfing the same individual in that year. The factors are called by the symbols of MacArthur (1931); a full description of them is given in his paper. The plants were grown in water culture in a greenhouse, and nine samples were taken at four-day intervals, covering a month of growth. Twelve plants were taken from each line at each time. Eight randomized blocks were used, but, as was seen in the previous paper (Fabergé, 1936), the sum of squares for blocks is not significant and need not be taken into account. Two plants were accidentally lost in sample 3 of line *P*, so that there are in all 429 degrees of freedom instead of 431. The analysis of variance is then as follows:

Degrees of freedom			
Lines...	3
Times	8
LT	24
Individuals	...	}	394 = Error
LI	...		
TI	...		
LTI	...		
Total...	429

Four measurements were taken on each individual plant; dry and fresh weight of both roots and shoots. They are very highly correlated, and give identical results; only the dry weight of shoots need be considered here. Logarithms to the base 10 of the weight are used in the computations, 0.1 mg. being a unit. A consequence of using a logarithmic metric is that the mean square becomes independent of the mean, and this supplies a *measure* of relative variability, in the same sense as Pearson's coefficient of variability.

It is with the analysis of the 394 degrees of freedom for error that we are concerned here. This is done by means of a 4×9 table (Table I).

Each item in this table is the sum of the squares of deviations from a sample of twelve. Each sample is determined from its position in the table with respect to the four lines and nine times. The marginal totals supply the required information.

The four line totals show that the two diploids *P* and *Q* are much more variable than the two tetraploids *R* and *S*. This difference is highly significant:

TABLE I*
Decomposition of error of 1934 experiment

Times	Lines				Total
	<i>P</i>	<i>Q</i>	<i>R</i>	<i>S</i>	
1	0.2756	0.3200	0.0655	0.1941	0.8551
2	0.3663	0.4148	0.2796	0.2016	1.2623
3	0.1440	0.1932	0.1569	0.1363	0.6307
4	0.1284	0.3078	0.2168	0.1213	0.7743
5	0.4485	0.5189	0.1726	0.1415	1.2815
6	0.6895	0.4916	0.0728	0.1297	1.3835
7	0.2860	0.3670	0.1197	0.3154	1.0880
8	0.3810	0.4397	0.1526	0.3179	1.2912
9	0.2855	0.2787	0.1322	0.1556	0.8521
Total	3.0048	3.3318	1.3688	1.7134	9.4188

* Numbers rounded off from computations made with more decimals. Hence some totals do not check in last figure.

TABLE II

Source of variability	D.F.	Sum of squares	Mean square	<i>z</i>	0.01 point in the distribution of <i>z</i>
<i>P</i> + <i>Q</i>	196	6.3366	0.0323	0.3654	0.1662
<i>R</i> + <i>S</i>	198	3.0821	0.0156		

The other classification, with respect to genetic constitution, only gives a very small difference which is not at all significant:

TABLE III

Source of variability	D.F.	Sum of squares	Mean square	<i>z</i>	0.05 point in the distribution of <i>z</i>
<i>P</i> + <i>R</i> +	196	4.3736	0.0223	0.0664	0.1175
<i>Q</i> + <i>S</i> dpos r y	198	5.0452	0.0255		

Turning now to the totals on the right-hand margin of Table I, it is clear that there is no general increase of error with time, on a logarithmic scale. We may now test whether the variations in this series of errors can be attributed to random sampling. The general test for the heterogeneity of a series of variances is given in an Appendix to this paper by Mr W. L. Stevens (p. 398).

The variance of the series of errors is:

$$\frac{S(e - \bar{e})}{8} = 0.0748.$$

This value is compared with the variance of a theoretical χ^2 distribution:

$$88 \left(\frac{9.4187}{394} \right)^2 = 0.0496$$

(a slight correction has been made because entry $3P$ is based not on 11, but on 9 degrees of freedom). The ratio of these two variances multiplied by 8 gives a χ^2 value of 12.064, based on 8 degrees of freedom. According to Fisher's table of the distribution of χ^2 , the probability of exceeding it by random sampling lies between 0.2 and 0.1. One may thus consider the variations of this column as being due to random sampling. Since logarithms were used, this result shows that the variability of the populations may be considered as being only the W_o component of Blackman's growth equation:

$$W_t = W_o e^{rt}$$

where W_t = weight at time t , W_o = initial weight and r = relative rate or efficiency index (Blackman, 1919).

Embryo weights of lines P, Q, R, and S

In the first sample of the 1934 experiment, the plants had about five times the weight of the embryos. It appeared important to see if the reduction of variability associated with the tetraploid condition could be found in the embryos themselves.

Fifty embryos of each of the lines P , Q , R and S were dissected out, dried and weighed individually. The methods are fully described in the previous paper (Fabergé, 1936). In addition to variability, it was desired to test the normalities of the distributions, in view of Lindstrom's results. Lindstrom, as was seen before, found that the positive skewness of the distribution of diploid fruit weights disappears in the tetraploid. The cubes and fourth powers, together with the sums and the squares of the observations were accumulated by the method of Progressive Digitizing on Hollerith punched card machines (Baehne, 1935). I am very much indebted to the British Tabulating Machine Company for letting me use their apparatus.

An analysis of the absolute variability, on an arithmetic scale, is given in Table IV.

The difference in *absolute* variability between the diploid and tetraploid embryos is not significant, though it is in the same direction as in the growing population. It is also clear that the greater part of the difference is due to the very high variability of the line Q .

It was seen in the previous paper (Fabergé, 1936) that tetraploid

embryos are about 30 per cent heavier than diploid ones, it is consequently necessary to use a measure of *relative* variability. I am much indebted to Mr W. L. Stevens for pointing out a method for getting an estimate of the variance of $\log x$ without having to recalculate the sums

TABLE IV

Analysis of absolute variability: embryo weights

	D.F.	S. of S.	M.S.	D.F.	M.S.	z	0.05 z
Total	196	94267	480.96				
P 2x	49	21888	446.69	98	540.82	0.1244	0.1662
Q 2x	49	31112	634.94				
R 4x	49	20459	417.53	98	421.09		
S 4x	49	20808	424.65				

of squares after transforming into logarithms. It represented a saving of time, since the necessary third and fourth powers were already available for the tests of normality. The numerical values of the terms of this transformation are given in Table V, where S_2 , S_3 , and S_4 represent the sums of the 2nd, 3rd and 4th powers of deviations from the mean respectively. The series of course continues, but only the first three terms are used here.

TABLE V

$$\underbrace{\frac{1}{\bar{x}^2} \cdot \frac{1}{n-1} \cdot S_2}_{P} - \underbrace{\frac{1}{\bar{x}^3} \cdot \frac{1}{n-1} \cdot S_3}_{Q} + \underbrace{\frac{11}{12} \cdot \frac{1}{\bar{x}^4} \cdot \frac{1}{n-1} \cdot S_4 - \frac{1}{4} \cdot \frac{1}{\bar{x}^4} \cdot \frac{n-1}{n^2} \left(\frac{1}{n-1} S_2 \right)^2}_{R}$$

P	0.047045	+0.005242	+0.004655
Q	0.063851	-0.014742	+0.011489
R	0.021886	+0.000244	+0.001032
S	0.027309	+0.002036	+0.001782

It will be noted that by taking the square root of the first term and multiplying by 100, Pearson's coefficient of variability is obtained.

The result of the transformation is given in Table VI. Under "growing population" are given the variances from Table I multiplied by $(\log_e 10)^2 = 5.30197$, since these were calculated on logarithms to the base 10.

TABLE VI

Lines	Embryos		Growing population		$V(\rho)/V(e)$
	D.F.	Logarithmic variance	D.F.	Logarithmic variance	
$P\ 2x$	49	0.05694	97	0.16436	2.886
$Q\ 2x$	49	0.06060	99	0.17868	2.949
$R\ 4x$	49	0.02316	99	0.07317	3.159
$S\ 4x$	49	0.03113	99	0.09172	2.947

In the last column is given the ratio of relative variability of the population to that of the embryos. From this it is clear that the varia-

bilities of the growing populations are very closely proportional to those of the corresponding embryos, though they are about three times as great. Thus the variability which, as was seen before, does not increase during growth, does so during germination.

The difference between the relative variability of diploid and tetraploid embryos, unlike the difference in absolute variability, is highly significant, as the following table shows:

TABLE VII

Lines		D.F.	Relative variability	z	0.01 pt. of z
$P + Q$	$2x$	98	0.05877		
				0.3861	0.2326
$R + S$	$4x$	98	0.02715		

The tests of normality on the embryo populations are given in Table VIII. g_1 and g_2 are used (Fisher, 1936); the standard errors appended to them are large sample standard errors.

TABLE VIII

Tests of normality: embryo weights

Line	g_1	S.E. of g_1	g_2	S.E. of g_2
P	-0.4932		-0.5936	
Q	+0.8772	± 0.3366	+0.2880	± 0.4381
R	-0.0725		-0.5314	
S	-0.4331		-0.2403	

There is clearly no consistent difference in skewness between diploids and tetraploids such as Lindstrom found in the case of fruit weights. It should be noted on the other hand that tests of skewness with fifty individuals are not very sensitive. Line Q alone departs significantly from normality. It is interesting to note that the outstandingly high absolute variability of this line vanished in a logarithmic metric, and that this is due to the correction introduced by the third power term in Table V. Had the coefficient of variability been used instead of the logarithmic variance, the measure of relative variability would have been affected by the skewness.

None of the four populations depart significantly from normality symmetrically.

1935 experiment

In the 1935 experiment ten populations were used, which may be classified as follows:

$$\text{Diploid} \dots \dots \left\{ \begin{array}{l} \text{Pure lines} \dots \dots \left\{ \begin{array}{l} A \\ I \\ J \end{array} \right. \\ F_2 \dots \dots \dots \left\{ \begin{array}{l} F \\ G \end{array} \right. \end{array} \right.$$

$$\text{Tetraploid} \quad \dots \quad \left\{ \begin{array}{l} \text{Pure lines} \quad \dots \quad \dots \quad \dots \quad \left\{ \begin{array}{l} B \\ C \\ D \\ E \\ H \end{array} \right. \\ F_2 \dots \quad \dots \quad \dots \quad \dots \quad \left\{ \begin{array}{l} B \\ C \\ D \\ E \\ H \end{array} \right. \end{array} \right.$$

Full descriptions of these lines are given in the previous paper. It need only be noted here that in the case of *G* and *H*, the same individual F_1 plant is the parent of both, and that *A* and *B* came from selfing the same individual in 1932.

The experiment is arranged in four randomized blocks. Eight samples at intervals of one week were taken, 12 plants from each line (three from each block). The first three samples came from a greenhouse, the remaining five from outside. Only the dry weight of shoots was recorded.

The theoretical structure of the experiment may then be represented as follows:

TABLE IX

Criteria of classification and their interactions	Degrees of freedom	Items appearing as such in the main analysis	Items making up absolute error	Items making up residual error
Lines	9	+	.	.
Times	7	+	.	.
Individuals	2	.	+	.
Blocks	3	.	.	+
LT	63	+	.	.
LI	18	.	+	.
LB	27	.	.	+
TI	14	.	+	.
TB	21	.	.	+
IB	6	.	+	.
LTI	126	.	+	.
LTB	189	.	.	+
LIB	54	.	+	.
TIB	42	.	+	.
LTIB	378	.	+	.
Total	959	.	640	240

It is the absolute error which supplies the information on variability. It is based on 640 degrees of freedom, and consists, as will be seen from the table, of *I* and of all the interactions into which *I* enters.

The analysis is carried out in essentially the same way as in the case of the 1934 experiment. A slight complication is introduced by the use of blocks which, unlike those of the 1934 experiment, are highly significant. Of the 11 degrees of freedom in each sample, three are due to blocks. Each entry of the decomposition of error table is the sum of squares based on the remaining 8 degrees of freedom. The decomposition of error is given in Table X.

This resembles the analysis obtained in 1934 very closely. It is clear from the right-hand column of totals that there is no general increase of

error with time on a logarithmic scale. But the variation in this column cannot be accounted for by assuming that it is the result of random sampling, as was the case in the 1934 experiment. The test, when carried out in the same way, gives a χ^2 value of 43.1561 for only 7 degrees of free-

TABLE X
Decomposition of error of 1935 experiment

Times	A	B	C	D	E	F	G	H	I	J	Total
1	0.53535	0.34089	0.43945	0.21799	0.54641	0.13425	0.23003	0.22113	0.03303	0.33970	3.03823
2	0.34959	0.32053	0.57519	0.09569	0.52286	0.20765	0.33430	0.11275	0.15083	0.61205	3.28744
3	1.06050	0.15995	0.44537	0.43001	0.43897	0.62372	1.33180	0.12104	0.05725	1.60560	6.28021
4	0.37193	0.08264	0.13118	0.04075	0.16493	0.35316	0.54804	0.10172	0.09952	0.06498	1.95885
5	0.10138	0.13716	0.07881	0.17507	0.27852	0.10927	1.04003	0.56744	0.11544	0.30671	2.90983
6	0.18404	0.41857	0.15603	0.27812	0.17632	0.11342	0.92087	0.33547	0.36146	0.06965	3.01395
7	0.78500	0.22968	0.21095	0.31651	0.16680	0.85123	0.13013	0.81581	0.27220	1.17267	4.95698
8	0.15377	0.26167	0.04905	0.30754	0.42499	0.51180	0.14836	0.61058	0.19737	0.17847	2.84360
Total	3.54756	1.95709	2.09203	1.86168	2.71980	2.90450	4.68356	2.88594	1.28710	4.34983	28.28909

dom, which is very significantly above expectation. No doubt this is due to uncontrolled factors inevitable in an outdoor experiment, which also covered a much longer period of growth, viz. 2 months. There is no reason to suspect the sampling technique adopted.

The comparison of diploid and tetraploid populations as regards variability is as follows:

TABLE XI

Variance within	D.F.	S. of S.	M.S.	z	0.01 z
Diploids	320	16.77255	0.052414	0.1880	0.1303
Tetraploids	320	11.51654	0.035989		

The difference is highly significant, and is in entire agreement with the results of the 1934 experiment.

It is of interest to compare separately the F_2 populations G and H , and also the pure lines A and B . The former pair comes from selfing the same F_1 individual, the latter from selfing the same individual in 1932. These comparisons are given below:

TABLE XII

Variance within	D.F.	S. of S.	M.S.	z	0.01 z
F_2 G	64	4.6836	0.07318	0.2421	0.2931
F_2 H	64	2.8859	0.04509		0.05 z 0.2072

TABLE XIII

Variance within	D.F.	S. of S.	M.S.	z	0.01 z
Pure line A	64	3.5476	0.05543	0.2974	0.2931
Pure line B	64	1.9571	0.03058		

In the former case, the difference is significant, although it does not reach the 0.01 level of probability. In the latter case the difference is highly significant.

The evidence from these two pairs of lines is more critical than from the remainder of the experiment, since they are very strictly comparable. It is clearly apparent that genetic constitution is capable of strongly influencing the amount of variability of a line.

It will be seen from the main decomposition of error table that the least variable of all lines is a diploid, the pure line *I*. This difference is highly significant, as the following test shows:

TABLE XIV

Variance within	D.F.	s. of s.	M.S.	<i>z</i>	0.01 <i>z</i>
Pure line <i>I</i>	64	1.2871	0.02011	0.3675	0.2005
Pure lines <i>A-H, J</i>	576	27.0020	0.04688		

No reason can be suggested for this apparently exceptional behaviour. The line *I* is a genetic stock called "Chinaman". It is known to have been cultivated since about 1860 in Australia, and is homozygous for several recessives.

The decomposition of error can also yield information on the comparative variability of pure lines and F_2 populations:

TABLE XV

Variance within	D.F.	s. of s.	M.S.	<i>z</i>	0.01 <i>z</i>
Pure lines	384	15.0953	0.03931	0.1354	0.1313
F_2	256	13.1938	0.05154		

Thus F_2 's are more variable than pure lines. To show that this is not due to any general increase of variability of F_2 's with time as compared with pure lines, the mean squares at each time are given below.

TABLE XVI

Times	Mean square	
	P.L.	F_2
1	0.0397	0.0354
2	0.0440	0.0368
3	0.0784	0.0786
4	0.0165	0.0365
5	0.0191	0.0624
6	0.0306	0.0483
7	0.0624	0.0614
8	0.0239	0.0530

Fruit weights

In the autumn of 1935 an investigation was undertaken which is similar in character to the one described by Lindstrom (1935) and discussed in an earlier part of this paper.

The experiment was not planned sufficiently long in advance for a proper field technique to be adopted; use was made of Dr Sansome's genetic populations which were intended for qualitative work only. It is confidently believed, however, that the main results are not in any way invalidated by this defect of experimental design.

Two F_2 populations were used—a diploid and a tetraploid. Both were obtained by selfing the same F_1 individual, the tetraploid being obtained by somatic doubling of part of that same individual. The parents were the stock "Chinaman" (line *I* of the previous experiment) and a pure line containing a new recessive factor making all parts of the plant very hirsute.

Sixty-eight plants of each of the two populations, growing in two long parallel rows, were used. The land was, so far as could be judged from the remaining crop, very uniform. Ten fruits were weighed from each of the 136 plants. In order to avoid bias, they were taken starting from the bottom of the plant and working outwards on each truss. Only fruits obviously injured, of which there were very few, were omitted in this order of picking. Fruit weights were recorded to the nearest gram.

An analysis of the results is given in Table XVII.

TABLE XVII
Analysis of variance of fruit weight

Variance	D.F.	2x		4x	
		s. of s.	M.S.	s. of s.	M.S.
Total	679	1162717.28	1712.40	323207.29	476.00
Between plants	67	325903.98	4864.24	138110.09	2061.34
Within plants	612	836813.30	1367.34	185097.20	302.45

Actual numbers, not logarithms, are used here.

It is clear that there is the same reduction of variability as was found by Lindstrom, and it is of the same order of magnitude. The total amount of variability is rather greater in our case than in his—a remarkable circumstance, since the parents of Lindstrom's F_2 differed much more in respect of fruit size than the ones used here.

In terms of the conventional coefficient of variability, the two sets of data are as follows:

	Lindstrom's data	Present data
2x	36.5	50.23
4x	29.4	40.53

An analysis of relative variability is given in Table XIX. By variability is designated the statistic

$$\frac{1}{x^2} \cdot \frac{1}{n-1} S(x-\bar{x})^2.$$

This corresponds to the first term of the transformation given in Table V, and its square root ($\times 100$) is the coefficient of variability.

TABLE XIX
Relative variability

Source of variability	D.F.	2x	4x	z	Points of z	
Total	679	0.2520	0.1704	0.1956	0.01	0.0893
Between plants	67	0.7158	0.7108	0.0035	0.05	0.2025
Within plants	612	0.2012	0.1043	0.3285	0.01	0.0941

The most striking thing in this table is that the entire reduction in variability is in the "within plants" component. The very small difference between plants is not at all significant, as the z tests show. This result has very important consequences on the interpretation of other data presented here.

These fruit measurements also show that polyploidy results in a reduction of fruit size, as has been found before by Jørgensen (1928) and Lindstrom (1935).

TABLE XX
Fruit weight

	Mean weight in gm.	Difference	Standard error
2x	82.44		
4x	53.85	28.59	2.83

It should be noted that although the mean fruit weight is about $16\times$ greater in our case than in Lindstrom's, the proportional reduction resulting from polyploidy is about the same.

DISCUSSION

It appears from the data presented that polyploidy consistently results in a diminished variability. The results of the growth-rate experiments clearly show that segregation of factors controlling growth rate does not occur to an appreciable extent. The populations may then be represented on a logarithmic metric as consisting of parallel lines:



and not of lines at an angle to one another:



In other words, the whole of the variation is in the W_0 component of the exponential equation

$$W_t = W_0 e^{rt}$$

which Blackman (1919) has applied to the growth of plants.

Significant differences between the variabilities of diploids and tetraploids are found on each occasion; it follows that this is a difference in the variabilities of initial weights, and not of growth rates. The embryo data fully confirm this, since it was shown that the lesser variability of tetraploids is already present there. The variabilities of the embryos were very closely proportional to those of the growing populations, though the latter were about three times greater. It is difficult to say, in the absence of exact knowledge about growth during germination, what the mechanism of this accentuation of variability is. It is reasonable to suppose that the greater part is due to differences in the time when germination begins. In the previous paper it was shown that tetraploids (having 30 per cent heavier embryos) germinate more slowly, but the data given here show that *within lines*, larger embryos in some way gain proportionately more.

If Lindstrom's explanation of the reduced variability of tetraploids is to be applied to this case, it would be necessary to assume that all genes determining total plant size have already finished acting at the time the seed is ripe. This seems very unlikely. The absence of any consistent change in skewness is also against Lindstrom's view.

The evidence from fruit weights is quite categorical. Since the difference in variability is only between *different parts of the same individual*, it cannot be due to genetic differences between individuals. This at once makes it impossible to apply Lindstrom's explanation, which requires tetrasomic segregation ratios and a dominance of quantitative genes. Lindstrom, as was already seen, had deduced his hypothesis from fruit weight data precisely similar to those given here, and which in fact very closely resemble his own quantitatively. It is particularly regrettable that the methods of Analysis of Variance were not used in Lindstrom's case, for it is only by separating the total sum of squares into two components, ascribable respectively to "within" and "between" plants, that the true nature of the variation can be demonstrated.

The almost complete absence of difference of variability "between" plants must mean that a bigger plant does not produce heavier individual fruits. It may of course produce more fruits. It was seen that F_2 populations were rather more variable than pure lines in the 1935 experiment. Much weight cannot be attached to this, since the populations were not in any sense comparable.

A priori there is no obvious reason why polyploidy should reduce the variability at all, and it is therefore reasonable to attribute both the diminished variability of whole plants and of fruits to the same cause. The analysis of the results on fruits clearly shows that the component of variability which is reduced by chromosome doubling is not due to genetic differences between the objects measured. We must therefore be dealing with non-heritable variation. If this applies equally to whole plants, the difficulty of assuming that all quantitative factors have already finished acting when the seed is ripe is removed, and the absence of any change in skewness accounted for. It has been customary, more particularly since Johanssen's classical experiments with beans (Johanssen, 1909) to divide all variation into two classes: genetic and environmental. Now, the kind of variation dealt with here, the extent of which is diminished by tetraploidy, is clearly not genetic. But to say that it is environmental requires a discussion of what is meant by environment in this case.

The decompositions of error show that, in the case of whole plants at any rate, one must not think of an environment acting throughout development to produce differences. There is no information in the data presented here as to when variability arises in fruits, but it is of interest to note that Houghtalling (1935) has shown that the size of tomato fruits is already fully determined at a very early stage (open flowers). Thus here again it would appear that there is no question of an environment acting continuously throughout development, but only during the formation of early meristems.

Genes determine the form and size of the organism. During their action, circumstances may arise under which two genes fail to have full effect, but four are sufficient for a complete or more complete action. It is suggested that a process of this kind will best account for the effect observed. In any case this must take place in the very earliest stages of development. The existence of such a mechanism of "strengthening" of gene action by the increase of the number of genes cannot of course be regarded as proved by the data presented here. It is merely put forward as plausible. The subject is too new, and the technique too little developed for a definite answer to be given without much more extensive investigations.

It remains to point out that the idea is not unrelated to Timoféeff-Ressovsky's treatment of the intensity of gene manifestation (Timoféeff-Ressovsky, 1934). The "intensity" of different allelomorphs is classified into "Penetrantz" or the probability of the gene producing a visible effect at all, and "Expressivität", which is the degree of its manifestation. Both are in any case highly correlated. In this terminology, one might say that either or both the Penetrantz and the Expressivität of quantitative genes is increased by an increase in their absolute number.

SUMMARY

1. Experiments described in a previous paper, comparing diploid and tetraploid tomatoes with regard to size and growth, are analysed from the point of view of variability.
2. New data on fruit weight in diploid and tetraploid tomatoes are given.
3. Tetraploids are found to be consistently less variable than diploids. When the weight of whole plants is considered, it is shown that this reduction of relative variability lies entirely in the W_0 term of Blackman's growth equation $W_t = W_0 e^{rt}$. In fruits, the reduction of variability is between fruits on the same plant, and not between different plants.
4. It is considered that this diminished variability cannot be accounted for by a direct genetic effect involving segregation, as had been suggested by other workers.
5. An alternative hypothesis is proposed. It is assumed that doubling the number of genes results in an increased probability of the action of quantitative factors. This is reflected in the greater physiological stability of early meristems.
6. The fact that tetraploidy results in diminished fruit size is confirmed.

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APPENDIX

HETEROGENEITY OF A SET OF VARIANCES

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We shall first consider a general problem, of which the test of heterogeneity of a set of estimated variances is a special case.

Let x_r , $r=1, 2 \dots k$, be k quantities normally distributed with known variances σ_r^2 about unknown means μ_r , i.e.,

$$x_r = \mu_r + \sigma_r \eta_r$$

where η_r is normally distributed about zero with unit standard deviation. Then it is required to test the hypothesis that the means μ_r are equal for all r .

If the hypothesis is true, an efficient estimate of the common mean would be

$$\begin{aligned} m &= \frac{\sum_{r=1}^k \left(\frac{x_r}{\sigma_r^2} \right)}{\sum_{r=1}^k \left(\frac{1}{\sigma_r^2} \right)} \\ &= \mu + \frac{\sum \left(\frac{\eta_r}{\sigma_r} \right)}{\sum \left(\frac{1}{\sigma_r^2} \right)}. \end{aligned}$$

Hence

$$\begin{aligned} \sum \left\{ \frac{1}{\sigma_r^2} (x_r - m)^2 \right\} &= \sum \left\{ \eta_r - \frac{1}{\sigma_r} \cdot \frac{\sum \left(\frac{\eta_r}{\sigma_r} \right)}{\sum \left(\frac{1}{\sigma_r^2} \right)} \right\}^2 \\ &= \sum_{r=1}^k \eta_r^2 - \frac{\left\{ \sum_{r=1}^k \left(\frac{\eta_r}{\sigma_r} \right) \right\}^2}{\sum_{r=1}^k \left(\frac{1}{\sigma_r^2} \right)}. \end{aligned}$$

Since the quantity $\sum \left(\frac{\eta_r}{\sigma_r} \right) / \sqrt{\sum \left(\frac{1}{\sigma_r^2} \right)}$ is a linear function of the η 's, with the sum of the squares of the coefficients equal to unity, it follows that

$$\sum \left\{ \frac{1}{\sigma_r^2} (x_r - m)^2 \right\} = \chi^2_{[k-1]}$$

i.e., is distributed in a χ^2 of $k-1$ degrees of freedom(1). The table of χ^2 may therefore be used to make a test of significance.

Application to a set of estimated variances. The hypothesis to be tested is that k sets of quantities x_{rt} ($r=1, 2 \dots k, t=1, 2 \dots n_r$) are distributed with a common variance σ^2 , respectively about the means $\mu_1, \mu_2 \dots \mu_k$, the values of which are unknown and need not be estimated.

For each set we have the estimate of variance

$$s_r^2 = \frac{\sum_{t=1}^{n_r} (x_{rt} - \bar{x}_r)^2}{n_r - 1}$$

which has a mean σ^2 , and variance $2\sigma^4/(n_r - 1)$.

Following the previous argument, we find that the best estimate of σ^2 is

$$\begin{aligned} & \frac{\sum_{r=1}^k \left\{ \frac{s_r^2 (n_r - 1)}{2\sigma^4} \right\}}{\sum_{r=1}^k \left(\frac{n_r - 1}{2\sigma^4} \right)} \\ &= \frac{\sum \{ (n_r - 1) s_r^2 \}}{\sum (n_r - 1)} = s^2 \end{aligned}$$

as would be found by the usual method. Hence

$$\sum \left\{ \frac{n_r - 1}{2\sigma^4} (s_r^2 - s^2)^2 \right\}$$

is distributed very nearly in a χ^2 of $k-1$ degrees of freedom. As however σ^2 is unknown, it is necessary to approximate by putting $\sigma^2 = S^2$, and we find that

$$\frac{1}{2S^4} \sum_{r=1}^k \left\{ (n_r - 1) (s_r^2 - S^2)^2 \right\}$$

is approximately in a χ^2 of $k-1$ degrees of freedom. Since this formula is sensitive to differences between the variances it is suitable for testing the hypothesis that the variances are equal.

It may be noted that in large samples the above test tends to equivalence to the one given by Pearson and Neyman (1931) based on the ratio of the weighted geometric and arithmetic means(2).

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FURTHER DATA ON GENETIC MODIFICATION OF RUMPLESSNESS IN THE FOWL

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INTRODUCTION

WE have recently described (1934) the inheritance of a mutant gene (Rumpless **Rp**) in the fowl which is responsible for changes in the structure of the caudal and synsacral regions. In our original material and in the experience of other investigators this gene behaved as a dominant. Fowls heterozygous for the gene lacked the free caudal vertebrae, one or two synsacral vertebrae, the fleshy rump, the tail feathers and the uropygial gland. This condition, which was likewise characteristic of the few known homozygotes examined, we referred to as complete rumplessness. The gene was shown to segregate normally, and both hetero- and homozygous embryos were of approximately normal viability.

After crossing with certain normal fowls a new class of heterozygote was observed in which all of the affected characters except the absence of synsacral vertebrae were modified toward the normal, although in varying degree. Such "intermediate rumpless" fowls showed some development of the rump, had tail feathers, caudal vertebrae (often fused), and some of them had uropygial glands, occasionally functional. The factors responsible for these modifications toward normal proved to be hereditary, and after selection and inbreeding among members of the modified stock, ratios approaching $\frac{1}{4}$ complete rumpless: $\frac{1}{2}$ intermediate rumpless: $\frac{1}{4}$ normal were obtained from *inter se* matings of intermediates; while from matings of intermediates by normals from the same stock there resulted chiefly normals and intermediates, with very few complete rumpless. This suggested that factors tending to produce normal development of the tail had been introduced from the normal unrelated stocks, and that in the presence of such factors the rumpless gene acted as a recessive. We supposed that such genes would be retained in normal stocks because of the protection which they conferred against abnormal development of the tail brought about by environmental vicissitudes of the sort exemplified by our "accidental" (non-genetic) rumplessness (Dunn & Landauer, 1925) and by Danforth's (1932) demonstration that rumplessness may be induced in normal fowls by subjection of early

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embryos to fluctuating temperatures. The effects of such genes on the dominance of rumplessness could thus be viewed as incidental to their normal physiological activity, and their selective value (and hence their retention or accumulation in normal stocks) as due not to the risks of recurrent mutation as Fisher had supposed but rather to the insurance which they provided against abnormal development from all causes.

NEW EVIDENCE

The evidence which we had obtained was incomplete in several respects, and we have endeavoured to remedy these defects by new experiments. These have been only partially successful, but since we shall not be able to continue our work on this problem we shall record the final data which permit decisions on three points.

(1) *Homozygosity of "modifying genes"*

We supposed that the genes modifying rumplessness toward normal were recessive and multiple and were transmitted both by intermediates and by normals of selected intermediate ancestry. "When complete homozygosity (of modifying genes) is reached, crosses of intermediates by normals of similar genetic constitution should segregate only for the intermediate and normal conditions and no completely rumpless fowls should be produced, i.e. complete rumplessness should have become recessive" (1934, p. 226). This test was made in 1935. An intermediate male was mated with normal sibs from the selected stock. The result was clear: the progeny consisted of 148 intermediates and 132 normals (adult descriptions). No complete rumpless progeny appeared. It is probable then that a sufficient concentration of homozygous "modifiers" had been reached to induce some normal development of the tail in every rumpless heterozygote. The same result might arise if a single dominant "modifier" were present in homozygous form in the intermediate male, but this is extremely unlikely: (1) because *inter se* matings of fowls which from their ancestry should be heterozygous for such a dominant (e.g. our mating 1933-1, Table IV, 1934) have not given the ratios of 3/4 intermediate to 1/4 complete rumpless expected on this assumption; (2) because of the gradual increase in the proportion of intermediates among all rumpless heterozygotes from 22 per cent in 1926 to 100 per cent in 1935. It is probable then that the modifiers are multiple and recessive and that many of them have become homozygous in the stock following selection

(2) *Homozygosity of completely rumpless fowls*

The second question left open was whether all complete rumpless fowls from *inter se* matings of selected intermediates are homozygous for the rumpless gene. This was required by our assumption that modification toward normal occurred only or chiefly in rumpless heterozygotes.

The test is not entirely decisive because of the increasing difficulty of classification as the intermediate class continues to approach more nearly to the normal. Eleven completely rumpless females (from *inter se* matings of intermediates) were tested by mating with a normal male from the same stock. There resulted seventy-nine intermediates and eighteen progeny classified as normal at 2-3 months of age. All except one of these "normals" had been recorded as intermediate or questionable at hatching. Three "normals" were dissected when adult. All had normal tail vertebrae, but each lacked one synsacrocaudal vertebra, as do the intermediates. This evidence, together with the observations at hatching, make it quite likely that the normals in this case were rumpless heterozygotes showing extreme modifications towards normal. Of the individual rumpless birds tested seven produced only intermediate progeny; four gave only intermediates and birds of the questionable "normal" or intermediate type (one gave twenty-three intermediates and two intermediate (?) and was certainly **RpRp**; one gave twenty-three intermediates and eight (?) and was probably **RpRp**; one gave seven intermediates, one intermediate (?) and one normal (?) and was probably **RpRp**; one gave four intermediates and one intermediate (?), an insufficient test). Although not decisive, the test shows that most completely rumpless fowls from matings of intermediates are probably homozygous **RpRp**. Complete rumplessness, which at the beginning of the experiments was characteristic of heterozygotes, is probably now expressed only in the homozygote.

(3) *Does modification occur only in heterozygotes?*

A related question was whether modification toward normal was confined to the heterozygotes. Our previous evidence suggested that this was so, since we obtained about 1/4 rumpless, 1/2 intermediate and 1/4 normal from *inter se* matings of intermediates. Two further generations of selection and inbreeding among the intermediates, however, have thrown doubt on the earlier assumption. The results of matings among intermediates during 1934 and 1935 are shown in Table I, compared with the results previously published.

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The proportion of fowls with the rumpless gene which show the intermediate condition has increased steadily with increasing concentration of "modifiers". If the "modifiers" affect only the heterozygotes the limiting value of this proportion should be 66 $\frac{2}{3}$ per cent; this is definitely exceeded in the 1934 and 1935 results. The proportion of fowls inheriting the rumpless gene itself (homozygotes and heterozygotes together) has not changed significantly from the 75 per cent expected, showing that the viability of homo- and heterozygotes relative to normal has remained constant and thus cannot account for the changed proportion of intermediates. It is thus probable that some of the homozygotes have also been modified toward normal. The descriptions of most of the rumpless fowls during the last two years were based on external appearance and palpation only. Several fowls included among the complete rumpless in Table I were dissected and found to contain anky-

TABLE I
Results of matings between rumpless intermediates
(from adult descriptions only)

Year	Rumpless	Rumpless inter- mediate	Normal	Total	% of inter- mediates among all rumpless	% with rumpless gene
1927-32	200	147	135	482	42.4	72.0
1933	62	152	74	288	71.0	74.3
1934	38	136	50	244	78.1	77.7
1935	17	124	54	195	88.0	72.3

losed and reduced caudal vertebrae. We believe that many of our apparently rumpless fowls have now some characters of the intermediate condition (remains of caudal vertebrae, etc.), and that complete rumplessness has practically disappeared from our stock, even in fowls homozygous for the rumpless gene. Coincident with this change has been a further modification of heterozygotes toward normal until it is now impossible to distinguish many heterozygotes from normals except by dissection and examination of the synsacro-caudal vertebrae.

The "modifiers" thus act both on hetero- and homozygotes. In similar concentrations they modify heterozygotes relatively more than homozygotes toward normal. In extreme cases they may even extinguish all external character differences between heterozygote and normal. Genes with such effects need not be regarded as specific modifiers of rumplessness or of relative dominance. They are apparently constituents of the normal gene constitution, tending to promote the normal development of an important part which is especially subject to variation

from different causes. No apparent effect of these genes on the normal type has been observed. They would be retained in the normal type because of their obviously favourable effect on development. Our previous evidence (1934) had shown that one of their effects was to render recessive such harmful mutations as rumplessness. It is now shown that their effects go beyond this, and tend to extinguish also the homozygous effect of the rumpless mutation. This may be most simply conceived as a by-product of their activity in producing the normal characters.

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DEVELOPMENT OF EYE COLOURS IN *DROSOPHILA*: STUDIES OF THE MUTANT CLARET¹

By BORIS EPHRUSSI² AND G. W. BEADLE

INTRODUCTION

A PREVIOUS account of eye transplantation experiments involving the eye colour mutant claret (*ca*, 3-100·7) of *Drosophila melanogaster* (Beadle & Ephrussi, 1936) reported that a wild-type eye disk grown in a claret host develops pigmentation phenotypically like claret. From this it was concluded that a diffusible substance, tentatively called *ca*⁺ substance, is involved in the development of a wild-type eye, and that this substance cannot be supplied by a *ca* host (or is supplied in reduced amounts). In repeating such experiments, it was found that the results were not always uniform; in some cases wild-type eyes grown in *ca* hosts gave eyes phenotypically close to wild type, while in others eyes phenotypically close to *ca* were obtained. Since these experiments were made with larvae of which the ages were only approximately known, it was assumed that the lack of uniformity might mean that we were working at a period of development which included a critical time for the action (or uptake by the eye) of *ca*⁺ substance, and that some experiments had been made before, and others after this time. Additional work has shown that this assumption was correct. It is the primary purpose of this paper to present the evidence bearing on this question.

TRANSPLANTS MADE AT DIFFERENT AGES

Wild type (from crosses of the inbred stocks Florida and Swedish *c*) and *ca* (stock obtained from out-crosses to wild type) larvae of approximately known ages were obtained from eggs laid during 2-hour intervals. Errors from eggs laid in advanced stages of development were minimized by discarding early hatching larvae.

The first experiment was made with larvae taken at 86-89 hours after egg-laying. The results (Table I) show that disks from male donors gave eyes close to *ca*, but that those from female donors gave eyes slightly darker, that is, deviating from *ca* in the direction of wild type. This would suggest that the disks from female donors were more advanced developmentally at the time of transplantation than were the disks from

¹ Work done at the California Institute of Technology.

² Fellow of the Rockefeller Foundation.

male donors. Subsequently, two series of experiments were made using larvae of the same culture dish at different times, at 75–78 hours and at 91½–94 hours. These two experiments therefore should be strictly comparable. The results are summarized in Table I. The phenotypic appearance of the implants in the 75–78-hour experiment was like that of *ca* controls (*ca* eye disks grown in *ca* hosts and examined at the same time

TABLE I

Data on the differentiation of wild-type eye disks implanted in claret hosts.

In this and Table II, under the heading "Number of individuals", are given the four sex combinations and the total in the order: female in female, female in male, male in female, male in male, and total.

Age after egg-laying (hours)	Number of individuals	Phenotype of implant
86–89	2, 2, 1, 2; 7	♂ implants <i>ca</i> ; ♀ implants slightly darker than <i>ca</i>
75–78	3, 2, 2, 1; 8	<i>ca</i>
91½–94	3, 1, 4, 2; 10	Slightly darker than <i>ca</i>

as were the implants of the experiment). The implants in the 91½–94-hour experiment were slightly darker than the *ca* controls. In this series there was no indication of a difference dependent on the sex of the wild type donor. Since small differences were being considered, and since the two experiments were made at different times, it is questionable whether there was any real difference in the results of the 86–89-hour and the 91½–94-hour experiments.

The controls for experiments made in connexion with ovary transplants (Table II) confirm the results of the experiments just described. These show that wild-type eye disks transplanted to *ca* hosts at 73½–

TABLE II

Data on implantation of wild type eye disks and ovaries into ca hosts.

Arrangement under heading "Number of individuals" same as in previous table

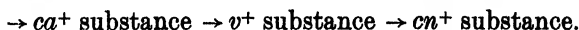
Nature and constitution of implants	Age of donor (hours after egg-laying)	Host		Number of individuals	Phenotype of implanted eye
		Constitution	Age after egg-laying (hours)		
+ ovary and + eye disk	85–88	<i>ca</i>	85–88	3, 1, 0, 0; 4	<i>ca</i>
+ eye disk	86–88½	<i>ca</i>	86–88½	0, 3, 0, 0; 3	<i>ca</i>
+ ovary and + eye disk	73–74½	<i>ca</i>	93–95½	3, 5, 0, 0; 8	<i>ca</i>
+ eye disk	73½–75½	<i>ca</i>	93½–96½	2, 6, 0, 0; 8	<i>ca</i>

75½ hours and at 86–88½ hours after egg-laying give eyes phenotypically similar to comparable *ca* control implants.

It is clear from the above experiments that if a wild-type eye disk is transplanted to a *ca* host before 80 hours after egg-laying, an eye will develop with *ca*-like pigmentation. From other experiments we know that if the above transplant is made shortly before puparium formation, the implant will develop a colour close to that of wild-type controls. It can be said, therefore, that the critical time with which we are dealing in the case of *ca* lies in that period of development reached some time after 80 hours and before puparium formation (about 106 hours at 25° C. for the wild-type flies used in these experiments). Presumably, before this time, a wild-type eye has received little or no *ca*⁺ substance from other parts of the body. By the time of puparium formation, however, a sufficient amount of *ca*⁺ substance has moved from the body to the eye so that removal of the eye to an environment unable to supply this substance (*ca* host) does not prevent its developing a colour like that of wild type.

These results indicate that certain conclusions arrived at in a previous paper may be erroneous. It was found that vermilion (*v*), cinnabar (*cn*), scarlet (*st*), and cardinal (*cd*) eye disks transplanted to *ca* hosts gave rise to eyes with *v*, *cn*, *st*, and *cd* pigmentation, respectively, that is, not-*ca*. These experiments were done with larvae the ages of which were not accurately controlled. From the experiments reported above on wild type in *ca* transplants, it is evident now that they might all have been made at or after the critical time for the action of *ca*⁺ substance. As a matter of fact, as will be pointed out below, the interpretation of the *v* and *cn* in *ca* experiments is probably even more involved.

From the results obtained by transplanting *v* and *cn* eye disks to *ca* hosts (Beadle & Ephrussi, 1936) it was concluded that the mutant *ca* is characterized by the absence of both *v*⁺ and *cn*⁺ substances. These are specific diffusible substances assumed to be related to *ca*⁺ substance in formation in a manner which can be expressed by the scheme:



Additional tests indicate that a *ca* host does not completely lack *v*⁺ substance but has a reduced amount as compared with wild type. Thus there appears to be a slight modification, in the direction of wild type, of a *v* eye disk grown in a *ca* host. The presence of *v*⁺ substance is more clearly shown by growing an *w*^a *v* (*w*^a-apricot) eye disk in a *ca* host; here the phenotype of the implanted eye approaches that of straight *w*^a. A difference in degree of change in *v* and in *w*^a *v* implants, when acted on by a limited amount of *v*⁺ substance, appears to be a general rule; as such,

it is discussed elsewhere (Ephrussi & Beadle, 1936). Preliminary experiments involving ca^2 , differing in origin from ca , indicate that a limited amount of cn^+ substance is formed in a ca^2 fly.

There is an obvious bearing of the evidence presented above on the general scheme which attempts to relate the three postulated diffusible substance. Discussion of this will be deferred.

TESTS FOR THE PRODUCTION OF ca^+ SUBSTANCE BY OVARIES

In an attempt to determine where ca^+ substance is produced in the body of a wild-type fly ovaries have been tested. Such tests can be carried out by making double transplants into ca hosts, a wild-type eye as a detector and, in the same host, the organ to be tested. Experiments in which ovaries were tested are summarized in Table II. These experiments were made with larvae of 73–74½ hours and 85–88 hours after egg-laying, that is, before the critical time of action of ca^+ substance. The results are entirely negative, showing that an ovary does not produce any appreciable amount of ca^+ substance under the conditions of the test.

SUMMARY

A wild-type eye disk transplanted to a claret host before 80 hours after egg-laying (25° C.) gives rise to an eye with pigmentation like that of claret. If the same transplantation is made shortly before puparium formation (about 106 hours after egg-laying), the resulting implant is phenotypically close to wild type. These results are interpreted by assuming that a specific diffusible substance (ca^+ substance) necessary for wild-type eye colour, moves from the body to the eye in a wild-type fly during some period between 80 and 106 hours after egg-laying, and that transplantation, after this critical time, of an eye from such a fly to a host which cannot supply the substance, does not modify the normal course of pigment development.

The relation of this critical time to experiments previously published is considered.

Tests for the production of ca^+ substance by the ovaries of wild-type flies gave negative results.

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THE PROBLEM OF DOMINANCE IN MAN

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(With Eight Text-figures)

SOME PECULIARITIES OF PATHOLOGICAL GENES IN MAN

THE opinion is rather widespread that the majority of mutant genes in man, as well as in most of the plants and animals which have been studied, are recessive. R. A. Fisher, in particular, emphasizes this view in working out his theory of the evolution of dominance. He cites *Homo sapiens* side by side with *Drosophila* and sweet peas as examples of species in which mutant genes are, as a rule, recessive. He does not deny that many *dominant* mutant genes in man have been described, but points out that this may be due to the fact that dominants in man are easier to discover than recessives. To prove this he calls attention to the fact that a comparatively large number of sex-linked recessive genes in man (which are discovered with relative ease) have been described, whereas we do not know as yet of any dominant genes in the sex-chromosome. Thus if most of the genes in the X-chromosome are actually recessive, then an analogous phenomenon should also be observed in the autosomes and only our imperfect technique prevents us from determining this. The same idea was pointed out by the author in 1929, and with this premise in mind then began a systematic investigation of pathological genes in man.

However, the hereditary diseases in man which the author and his co-workers have studied for the past six years have all proved to be *dominant*, with more or less limited penetrance. This was true for Basedow's disease (Levit *et al.*¹), ulcer ventriculi (Levin & Kuchur), leukaemia (Ardashnikov), eunuchoidism (Likhziemr *et al.*), bronchial asthma (Malkova), paroxysmal tachycardia (Ryvkin, Gassko) and apparently also for pernicious anaemia (Presnyakov), hypertonus (Ryvkin, Malkova and Kantonova, unpublished data) and other diseases.

¹ In this paper the authors advanced the preliminary hypothesis that the genes for goitre and for the classic Basedow's disease are dominant, and that the gene for toxic struma is recessive. Later extensive research of I. A. Ryvkin (unpublished data) confirms the conclusions regarding the first two diseases but throws some doubt as to toxic struma.

In testing the dominance of the mutant genes in the above-mentioned cases it is necessary to know which of the allelomorphs should be called mutant, and also which should be called dominant. The first question cannot always be answered, particularly when we deal with genes which do not produce any pathological effect. For example, we cannot say which of the allelomorphs that determine the blood groups in man is the normal gene and which is the mutant. The same is true of the gene for the taste reaction to phenyl-thio-carbamide and of other genes. However, it is another matter when we deal with pathological genes. There can hardly be any doubt that the mutant is the pathological gene in the great majority of cases.

Of no less importance is the second question. When may a gene be recognized as dominant? It should be recognized here that we hardly ever know how new mutant genes which produce a pathological effect in the heterozygote manifest themselves in the homozygote. Not knowing this, we cannot call them dominants in the strict sense of the term, since this designation presupposes that the homozygote (**AA**) does not differ from the heterozygote (**Aa**) in its phenotypic expression. On the other hand, the fact that the mutant gene produces a pathological effect when in heterozygous condition proves that it has some degree of dominance, at least, and that this degree of dominance is of practical consequence. The author proposes, therefore, the term "conditionally dominant" for those genes which give clear pathological expression in the heterozygote and whose homozygote is as yet unknown. This group of "conditionally dominant" genes should be considered as in part a provisional one. In time, as the phenotypic expression of the homozygote becomes known, this group will naturally be converted into complete dominants (probably the minority), intermediates, and probably some others.

There is some degree of probability that for some of the cases referred to here the homozygote is lethal. This inference may be drawn from the genetics of other species, where it is quite often observed that genes producing pathological effects in the heterozygote are lethal in the homozygote. This would provide a possible explanation of our cases in which the mating of two affected persons failed to produce in offspring a more serious form of the disease.

Still more important is the question, on what grounds have we established the "conditional dominance" of the genes in question? As criteria of dominance or recessiveness we have taken the following: (1) The percentage of inbreeding among parents who produce affected offspring (as is known, this percentage is increased in cases of recessiveness).

Although we studied comparatively rare diseases, in some cases extremely rare ones, in no case did we discover a percentage of inbreeding higher than that which exists in the normal population. (2) The ratio between affected and healthy persons among the various types of relatives. First we should compare the incidence of affected sibs with the incidence of affected children or parents. In the case of recessive characters the greater incidence of affected individuals among sibs as compared with children or parents is well known. But such a condition was never observed in our material.

Of particular significance is the fact that for the above-mentioned diseases we found pedigrees manifesting the disease during three, four and even more generations. The supposition of recessiveness naturally requires that in all cases of the latter type the matings were between the homozygote (aa) and the heterozygote (Aa); this is extremely un-

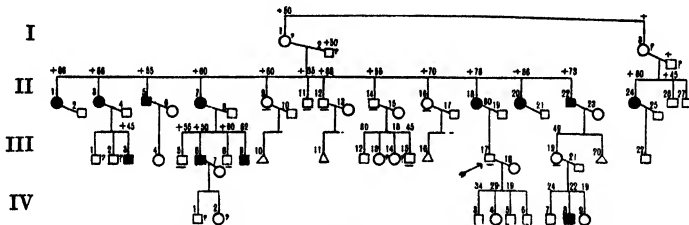


Fig. 1. Paroxysmal Tachycardia. Ryvkin's case. \square \circ Definitely unaffected. Δ Both sexes. —..... Existence of more children unknown. + Dead. Figures above horizontal lines indicate age.

likely in so far as cases of this kind have taken place in the absence of inbreeding, and often even in matings between persons who are either of different nationalities or who were born in localities far removed from one another. The objection may be raised that in groups in which given genes attain a relatively high concentration, pedigrees of the above kind should quite often be observed. But this objection does not hold for cases in which we deal with extremely rare diseases. Thus, the transmission of a very rare disease for a number of generations in the absence of inbreeding (see for example Fig. 1, concerning paroxysmal tachycardia, observed in our Institute by I. A. Ryvkin) can hardly leave a doubt that we are dealing with a "conditionally dominant" gene.

Special consideration should also be given to the question of the polyfactorial nature of the character, i.e. as to whether the latter may be conditioned by two or more rare genes acting jointly. However, where information can be obtained not only of near relatives but also of distant relatives, this possibility may be excluded. As an example we present

the data of Levit & Pessikova for diabetes mellitus (analysis of 222 pedigrees, see Table I):

TABLE I

Type of relationship	Unaffected	Affected	% affected
Parents	397	18	4.34
Sibs	445	16	3.59
Children	33	1	2.94
Grandparents	235	5	2.08
Uncles, aunts	309	12	3.74
Nephews, nieces	58	1	1.73
Cousins	220	2	0.91

As this table indicates there are approximately equal percentages of affected parents, sibs and children (the difference between them can easily be explained as a difference in age¹); approximately half as many grandparents as parents (the somewhat higher percentage of affected aunts and uncles may be explained by the poor reproductivity of diabetics); approximately half as many nephews and nieces as children; and about one-fourth as many cousins as sibs. These proportions are characteristic of monofactorial dominant heredity when the pathological gene is sufficiently rare. If, on the contrary, we were dealing with a polyfactorial disease the decrease in the incidence of affected individuals among more distant relatives would proceed at a far more rapid rate (for example, if diabetes were due to the combined action of two rare dominant genes, the percentage of affected grandparents should be about one-fourth the percentage for the parents, etc.).

The question arises as to whether or not the fact that all the mutant genes investigated by us happened to be dominants was due to chance. This is hardly possible since we are dealing with a comparatively large number of diseases. Moreover, a critical review of the literature has convinced us that the phenomenon observed by us is characteristic for man in general. In this connexion it should be emphasized that it is exceedingly difficult to study the literature on the genetics of man from this standpoint. The difficulties are of two kinds. First, the literature itself is immense. Second, the greater part of it concerns investigations carried out by inexact methods. Hence the available literature, and in particular the conclusions of individual authors, must be used with great caution. For this reason we chose four collected works: Davidenkov (nervous diseases), Siemens (skin diseases), and Franceschetti and Waardenburg (both eye diseases). We approached the facts presented in these summaries critically, and did not recognize as recessive and

¹ Penetrance of diabetes increases with age.

dominant genes nearly all that the authors designated as such. We excluded particularly those diseases which were described as casual observations and especially such of these as gave contradictory results leading the authors to infer dominance in certain cases and recessiveness in other cases of apparently the same disease. We, therefore, fixed our attention primarily on those diseases concerning which there are systematic investigations. In many cases we became familiar not only with the detailed data presented in the summaries enumerated above but also with the original investigations before we finally decided to interpret the hereditary disease in question as "conditionally dominant" or recessive. This critical examination of the data in the literature resulted in the following classification for autosomal genes:

Conditional dominants	Recessives	Intermediates
38	14	3

These facts agree with our own findings and confirm our hypothesis that *the majority of mutant pathological genes in man are "conditionally dominant"*.

It should be emphasized once again that neither we nor the authors whose data we used made any special selection of dominant diseases. As a matter of fact a certain unconscious selection probably exists in the literature, but not because the authors concentrate their attention on "conditionally dominant" diseases. At first glance this sounds paradoxical, for it is usually supposed that as a consequence of man's small reproductivity it is more difficult to study recessive than dominant diseases. It seems to me that the contrary is true, namely, that recessive genes are easier to investigate than dominants, since increased incidence of inbreeding is usually already to be observed in the first few families which the investigator encounters. Moreover, there is a marked prevalence of affected sibs with an almost complete absence of other affected relatives. Then again, the penetrance of the recessive in the homozygotes is usually far better than the penetrance of "conditional dominants" in the heterozygotes. This is very important in view of the difficulties that poor penetrance raises in genetic analysis. Possibly all these conditions have influenced the selection of material previously studied (how otherwise can one explain the fact that such comparatively insignificant diseases as albinism, amaurotic idiocy, alkaptonuria and others have been so well investigated, while till recent times there has been a lack of studies of the genetics of such significant diseases as stomach ulcer, Basedow's disease, etc.?). At any rate, there is no doubt that there was no selection in favour of dominant diseases for the investigations.

From the data presented, then, we are led to conclude that there is a definite preponderance of "conditionally dominant" pathological traits in man.

A second trait, no less characteristic than dominance, is the *extreme variability (poor penetrance) of most pathological mutant genes in man*. We may say with more or less certainty that penetrance varies, in the diseases investigated by us, from a fraction of 1 per cent (leukaemia, pernicious anaemia) to 10–20 per cent and occasionally higher. The same poor penetrance is also to be seen when we make a careful study of the literature. Conditionally dominant genes give especially convincing evidence on this point when they are very rare. The skips found in the latter cases, especially where the disease is transmitted for several generations without inbreeding, leave no doubt that we are dealing here with a failure of penetrance (see for example Fig. 1). Recessive genes, on the contrary, probably show, as a rule, good penetrance in the homozygotes.

The following case is of interest in connexion with what has been said. Hogben (1932), using material gathered by Bulloch, came to the conclusion that diabetes insipidus is due to a good dominant gene. But from the point of view stated above we were in doubt about this, and so the author, together with L. N. Pessikova, made a study of this disease in their own material. The study clearly demonstrated that the gene for diabetes insipidus is not a good dominant gene. Among the twelve systematically gathered cases of diabetes insipidus included in this study only two were familial, and even these did not show perfect penetrance. Moreover, there are skips recorded in the material on which Hogben himself bases his conclusions (the cases of Wile, Case and Clay).

In this connexion the danger must be emphasized of formulating genetic generalizations on the basis of data taken from the literature, for such data are often "selected data". Thus all the cases gathered by Bulloch are selected ones, in that they deal with "interesting cases", that is, with cases in which the phenomenon of inheritance is expressed most distinctly. Our material, on the other hand, differs from that used by Hogben in that our investigation was systematic: that is, familial as well as non-familial cases were taken without selection and subjected to investigation.

A third characteristic is suggested by the study of pathological genes in man. This is that recessive diseases have a tendency to manifest themselves at an earlier age than "conditionally dominant" diseases. We frequently encounter the latter in persons of fairly advanced age, and

sometimes the manifestation is seldom to be found before the end of the reproductive period—a circumstance often observed in diabetes mellitus, for example, as well as in other diseases.

Such are the rules which may be formulated on the basis of the data obtained in our investigations. But here another question arises. If they are correct, and if, in particular, the basic thesis is correct, that most pathological mutant genes in man are “conditionally dominant”, then how is all this to be reconciled with the statement cited above that almost all genes localized in the X-chromosome are recessive? With this question in view we have conducted a detailed study of the latter problem. Our results show that just the reverse conclusion is the correct one; namely, that only a small minority of genes which can be proved to be sex-linked have given definite evidence of being recessive. We may now proceed to examine the evidence on this point.

SEX-LINKED GENES IN MAN (IN RELATION TO THE PROBLEM OF DOMINANCE)

About thirty genes located in the X-chromosome of man have been described. The large majority of these genes have been considered to be recessive. When the material is reviewed in detail, however, one is surprised at how uncritically the authors consider their cases, both in regard to the evidence for sex-linkage, and in regard to that for recessiveness. The criterion usually employed for sex-linkage consists in the manifestation of the disease only or chiefly by males and its transmission by females. Detailed proof should hardly be necessary to demonstrate that this criterion alone is inadequate, for autosomal dominant genes *limited* (entirely or in part) to the male sex meet these conditions and behave in very much the same way. To be sure, the authors frequently point out that the *non-transmission of the character from father to son* is another and necessary criterion for recognizing a sex-linked gene. This criterion can be correctly applied, however, only when there is a large enough number of sons of a male carrier of the character. But strangely enough no attention has ever been paid to this condition.

The following case is presented as an illustration. Lenz (1932), in discussing the genetics of nystagmus, cites a pedigree of Nodop, which is supposed to illustrate the sex-linked type of inheritance. This pedigree is given by Lenz in an abbreviated form. But it is characteristic that Lenz abbreviated just that part of the pedigree in which a healthy son was born of an affected father, although this was really important contributory evidence for his own hypothesis.

The treatment of the genetics of Pelizaeus-Merzbacher disease offers another example of exceptionally careless handling of this problem. This is an extremely rare disease of the nervous system and, so far as I know, its genetics is based on observations of only one family (see Fig. 2). But this family record contains no mating of an affected male, and consequently no evidence of non-transmission from an affected father to his sons. Hence, as a matter of fact, it offers no proof of the presence of sex-linkage in this case. We might just as well assert that it is a case of autosomal inheritance. But let us assume that the gene in this case is actually located in the X-chromosome. Then why should it be regarded as recessive? As is indicated in Fig. 2, there are two affected females in this case, who obviously cannot be regarded as homozygotes. Therefore, even if we regard this as a sex-linked gene, it should be treated as "conditionally dominant", and perhaps partially limited to the male sex.

A large number of similar examples may be cited.

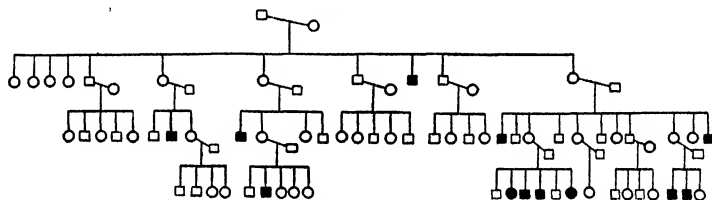


Fig. 2. Pelizaeus-Merzbacher disease.

All of the foregoing indicates the need for a reconsideration of the material presented in the literature as recessive sex-linked inheritance in man.

The criterion we have used consists of the absence of the trait in question among the sons of fathers carrying it. However, we have treated as sex-linked only those cases in which the sons of affected fathers numbered not less than nine, for only in these cases is the probability of such a result on the basis of autosomal inheritance (which we will call PA) sufficiently small $(1/2^9)^1$. Now in order to affirm the recessiveness of a gene already proved to be sex-linked we should have more than this criterion as well as more than the absence of this trait in female heterozygotes in one or several observed families. The latter condition is inadequate, because the absence of affected females may result from occasional non-expression of the dominant gene in a limited number of female heterozygotes, which may be either accidental or due

¹ The probability $1/2^9 = 0.00195$ corresponds approximately to that accepted in genetics as statistically significant (3σ).

to a partial limitation of the trait to the male sex.¹ To prove that a sex-linked gene is not "conditionally dominant" but strictly recessive, the thorough investigation of a large population is necessary. In that case, the ratio of the male to the female bearers of a recessive trait should be as $q : q^2$. This naturally follows from the distribution of sex-linked genes in the population, which, as is known, may be expressed by the formula $pA : qa$ (in males) and $p^2AA : 2pqAa : q^2aa$ (in females). If the proportion of females is greater than q^2 we may conclude that the sex-linked gene is partially dominant, manifesting itself not only in female homozygotes, but partly also in female heterozygotes. When the proportion of females is less than q^2 we may assume partial limitation to the male sex, i.e. partial non-expression in females. In these cases, however, we assume that there is no differential viability—a condition not to be taken for granted.

However, investigations of this kind have been made only in relation to colour-blindness. Other sex-linked genes have not been studied from this point of view. For this reason alone they cannot be strictly classified as recessive. If this is too rigorous a requirement, then the minimum requirement which a sex-linked gene must fulfil to be classified as recessive is that at least one affected homozygous female must be known (with the absence of the latter trait in female heterozygotes).

The necessity of the latter requirement follows from the possible complete limitation of given traits to the male sex. In cases in which sex-linked genes are wholly limited to the male sex, it is impossible to differentiate dominants from recessives. I propose calling such genes "indeterminates". It should be emphasized that these genes are "indeterminates", not because we know so little about them, but because the question of whether they are dominants or recessives is meaningless. Along with them we must also provisionally class as "indeterminate" all sex-linked genes in respect to which the data are still insufficient to show that they can express themselves at all in females.

From the above point of view the author has found that in the case of only twelve of the genes described as sex-linked can the evidence for sex-linkage be considered as fairly conclusive. In respect to dominance these genes may be divided into the following four categories, with the behaviour of the genes in females as the criterion for this division.²

¹ We have some evidence that apparently the "conditionally dominant" pathological genes are limited more frequently to the male sex than to the female. This still awaits an explanation.

² The symbols designate: -- absence of trait, - + weak expression of trait, + + definite presence of trait.

Classification of genes	Heterozygote	Homozygote
Indeterminate	--	--
Recessive	--	++
Intermediate	-+	++
Conditionally dominant	++	?

We may now proceed to a separate consideration of each of these categories.

A. *Indeterminate genes*

Here are included those genes whose manifestation has not yet been observed in either heterozygous or homozygous females. These comprise:

(1) Haemophilia. Strangely enough this gene is always treated as recessive. However, we do not know, as yet, of the existence of this trait in females, though matings from which we would expect homozygous females have apparently taken place. Consequently, the complete limitation of this gene to the male sex is to be presumed. The data of Schlössmann (1930) concerning the intermediate expression of haemophilia in female heterozygotes (mild bleeding, slight retardation of blood coagulation and other symptoms) could not be confirmed by a test investigation made in our institute by Freidberg.

(2) Hemeralopia, accompanied by myopia (Varelmann, Kleiner and others). A very rare anomaly.

(3) A peculiar form of juvenile glaucoma, accompanied by coloboma and ending with blindness. This character was described by Frank-Kamentzky in the former Irkutsk district. He observed three families.

The comparatively large number of indeterminate genes (one-fourth of the total number of sex-linked genes) may probably be explained by the small amount of material investigated in the given cases. That is, the inclusion of these genes in this category is still for the most part provisional. The possibility is not excluded that when more cases have been observed the characters here mentioned will be found in heterozygous females, so that some of the characters described here will then be of the "conditionally dominant" type. But if any of the characters are observed only in the female homozygotes, then the corresponding gene will be classified as recessive.

B. *Recessive genes*

These affect homozygous females but, so far as is known, are unexpressed in the heterozygote. The known cases are:

(1) Megalocornea. The most convincing cases are Kayser (1914) and Grönholm (1921). In the latter case, this trait was observed in two

female homozygotes. We cannot be certain that megalocornea is not expressed in the heterozygote, since it is a very rare disease.

(2) *Ichthyosis vulgaris*. A total of ten cases, in which inheritance can be explained as sex-linked, have been described (see Orel, 1929). The affected males in five of these cases had no sons at all. The PA of the others equalled respectively $1/2^2$, $1/2^5$, $1/2^6$, $1/2^7$. In the case of Csörsz¹ there was a mating between an affected male and a heterozygous female. Among the offspring there were two affected females (apparently homozygotes). However, we cannot be certain that in the case of *ichthyosis vulgaris* there is complete recessiveness because the number of investigated cases is small. Moreover, it should be pointed out that this disease is chiefly inherited as a dominant autosomal trait. Hence, we cannot be sure that these ten cases are due to a sex-linked gene.

C. *Intermediate genes*

The genes referred to here are expressed in the homozygote as in group B, but differ by being also expressed to a degree in heterozygous females.

These comprise:

(1) Colour-blindness, the intermediate expression of which in female heterozygotes was recently shown by Wieland (1933). The question still remains as to whether the various forms of colour-blindness are due to different allelomorphs of one gene or to different genes in the X -chromosome. Besides, there are relevant cases of high expression of colour-blindness in individual female heterozygotes (see Wieland). This gives ground for considering the question of transferring colour-blindness from this group to the group of "conditionally dominant" genes.

(2) *Keratosis follicularis spinulosa*. A case of this kind (Fig. 3) was described by Siemens (1925). It should be pointed out that since the PA in this case is equal to only $1/2^7$, this case is not very convincing from the viewpoint of sex-linkage. However, Siemens treats also the case of Laméris, in which the PA is equal to $1/2^3$, in the same manner. These two cases taken together give adequate ground for regarding this character as due to a sex-linked gene. A milder form of the disease in female heterozygotes than in males was observed in Siemens's case. Hence we include this character in group C. However, as we do not yet know the phenotype of the homozygote for this trait, we cannot exclude the possibility that in the future (if the homozygote proves indistinguishable from the heterozygote) this character will prove to belong to

¹ Cited from Orel.

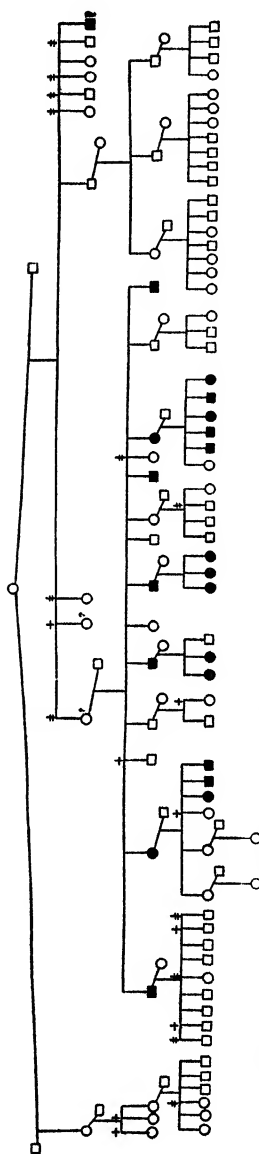


Fig. 3. Keratosis follicularis spinulosa, Siemann's case. + Died. • Mild form of disease.

the group of dominant sex-linked genes, perhaps with an expression limited, in degree, in females. Hence, this gene may be more accurately called "conditionally intermediate".

Summing up the above we may observe that the two genes which we classified in this group differ from one another, colour-blindness being closer on the whole to the recessive type, and keratosis follicularis to the dominant.

D. Conditionally dominant genes

Here we include those genes which have a definite effect, at least in certain cases in heterozygous females. The behaviour of these genes in the homozygous condition is not yet known.

These comprise:

(1) Leber's disease. A number of cases in which this is present in female heterozygotes have been described.

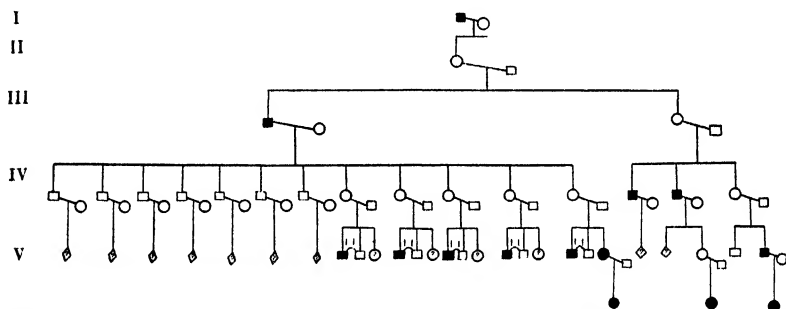


Fig. 4. Thadani's case (reconstructed by author). Anidrosis, adentia and hair defect.
 ? Number of siblings unknown. $\frac{1}{2}$ Ratio of affected to unaffected. ● Mild form.

(2) A complicated anomaly, including anidrosis, adentia, and hair defects. Darwin mentions one such Hindu family. Thadani (1921, 1934) has apparently described this same family twice (Fig. 4). Roberts (1929) described a similar case in U.S.A. Inbreeding was absent in both these cases, and this gene was found to be expressed in some of the female heterozygotes (a milder form in the case of Roberts).

Some other cases of this anomaly have been reported in the literature and have been collected by Cockayne. Some of these cases (Guilford-Atkinson, Cunningham, Kerley, Cockayne, Goeckermann, and others) also show some affected females. Cockayne distinguishes these from the other cases of the "recessive sex-linked type", as if they formed a separate class of cases showing dominance. We believe it probable that these all represent the same class of cases of conditionally-dominant with sex-

linkage, and in support of this note that there is not one case even among the pedigrees showing dominance, of an affected male having affected sons. The fact that when females are affected they usually have a more "attenuated form" of the anomaly agrees with this interpretation (it will be explained below why this should usually occur in sex-linkage).

(3) The absence of the central incisors. Only one case has been described (Huskins, 1930). The *PA* was rather small¹ in this case (see Fig. 5), and the character was expressed in females.

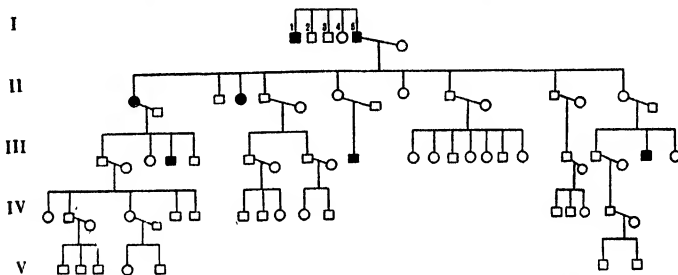


Fig. 5. Absence of central incisors. Case of Huskins. ● Mild form.

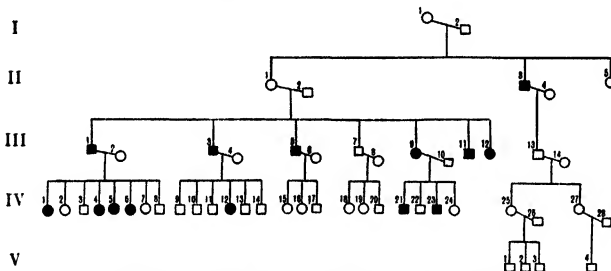


Fig. 6. Nystagmus. Waardenburg's case.

(4) Nystagmus. The genetics of this trait is very complex and as yet not thoroughly studied. In the literature nystagmus conditioned by a recessive sex-linked gene and that conditioned by an autosomal dominant are differentiated from one another. Lenz (1932) considers them as two allelomorphs (recessive and dominant) of one sex-linked gene. Moreover, without advancing any reasons he assumes the existence of a third allelomorph of recessive type, which simultaneously causes isolated albinism of the eye. I do not consider Lenz's arguments convincing, because he denies the autosomal inheritance of nystagmus while we have many cases of transmission from father to son (we have observed

¹ In 1, 1 there were six sons, who had many healthy offspring (footnote of the author in describing his case).

several cases of this type). Nor do I find convincing the differentiation of recessive sex-linked from dominant autosomal nystagmus, since among the pedigrees illustrating the second mode of inheritance we often find a very low PA (see, for example, of Waardenburg's case, Fig. 6; Hemmes treated this case as autosomal, whereas the PA here is equal to $1/2^9$). Moreover, in pedigrees usually considered as recessive sex-linked, there are cases of penetrance of the trait in female heterozygotes (see, for example, Engelhard's case). The case of Hemmes (Fig. 7) is especially odd in this sense. The author interprets it as a case including two types of inheritance: autosomal dominant (progeny 1, 1) and recessive sex-linked (progeny 1, 3). But it is rather clear that there are no grounds for such an interpretation, and that we have here a typical case of conditionally dominant sex-linked inheritance (PA is equal to $1/2^{11}$). Even if one agreed with Hemmes's interpretation, one would nevertheless have to regard the inheritance in progeny 1, 3 as being not recessive sex-linked but conditionally dominant sex-linked (due to the presence of an affected heterozygous female in 1, 3).

Considering the above, I am inclined to believe that the data prove the existence of at least two forms of primary nystagmus, one due to a conditionally dominant sex-linked gene of a phenotypically varying type (especially in females), and the second due to an autosomal dominant gene. The first form of nystagmus would include those cases previously described as sex-linked recessive, and in addition some of those cases which were previously described as autosomal dominants, and which have a sufficiently low PA (as, for example, the case of Waardenburg, presented in Fig. 6). In general this character demands further investigation. But at any rate the existence of a conditionally dominant sex-linked form of nystagmus can hardly be disputed.

(5) Retinitis pigmentosa. This character is usually inherited autosomally (apparently sometimes as a dominant and sometimes as a recessive). At the same time, McQuarrie recently described a case (Fig. 8) which makes sex-linked inheritance extremely probable for individual pedigrees (PA equal to $1/2^{14}$). The old cases of Usher and others, which were also interpreted as sex-linked but which were not convincing because the PA was too high, become more probable in the light of this observation.¹ In addition to the case presented in Fig. 8 there is at least one affected female, which gives ground for considering the character

¹ It should be emphasized that this case of McQuarrie's, the first case of retinitis pigmentosa which could be interpreted with certainty as sex-linked, proved to be incompletely recessive.

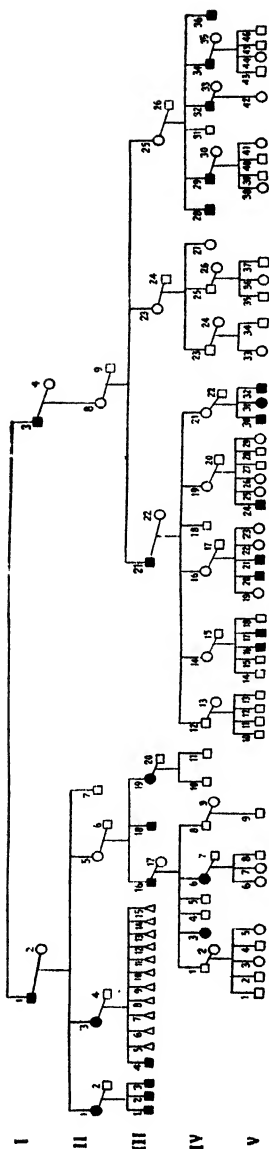


Fig. 7. Nystagmus. Hemmes's case.

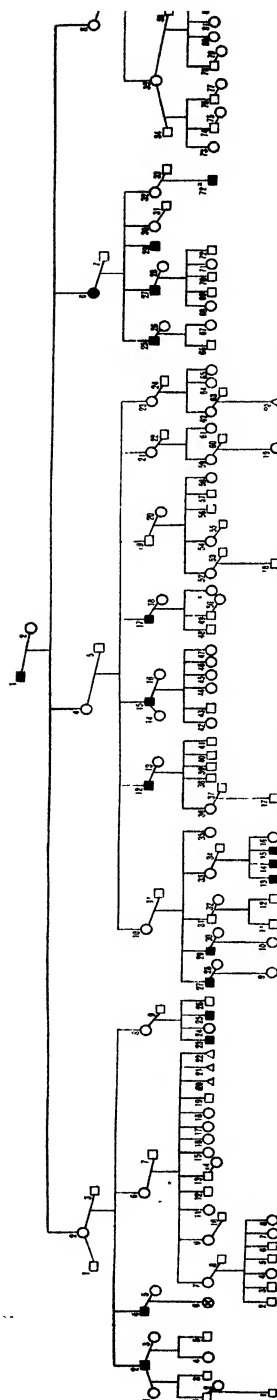


Fig. 8. Retinitis pigmentosa. Case of McQuarrie. ⊗ Blind, probably due to another cause.

in question as conditionally dominant. Supplementary investigation of the existence of conditionally dominant sex-linked forms of retinitis pigmentosa is required before a final conclusion is reached.

None of the other characters described as sex-linked satisfy our criterion of having a PA as low as $1/2^9$.

Thus, it is seen that the supposition regarding recessiveness of the great majority or even all (Fisher) of the sex-linked genes in man is incorrect. The contrary is rather the case. Recessive genes are actually in the minority among sex-linked genes (2 out of 12, and if colour-blindness is provisionally added to group B, then 3 out of 12).

It may be repeated that it is much easier to recognize recessive than dominant genes located in the X-chromosome, since the latter may easily be confused with autosomal genes.

The problem of the more marked expression of the genes in group D (and possibly also A) among males, and of reduced expression among heterozygous females, requires special consideration. This may be explained simply by the fact that the effect of a sex-linked gene in the male is about equal to the effect of this gene in the female homozygote (as found in *Drosophila*). On the other hand, the expression of a conditionally dominant sex-linked gene in a female heterozygote must naturally be comparable to the expression of a conditionally dominant autosomal gene in a heterozygote of either sex. But in this respect we see in both these groups (that is, in the autosomal as well as in the sex-linked genes) a similarly low frequency of expression of conditionally dominant genes in the heterozygote.

Summing up, we come to the conclusion that the data in regard to sex-linked genes not only do not contradict the hypotheses suggested here for autosomal genes in man, but, on the contrary, *confirm* these hypotheses.

DISCUSSION

It has become generally recognized in recent years that wild genes (normal allelomorphs) did not always so generally dominate over their mutant allelomorphs as they do now, but that at least some of them became dominant through natural selection. There is a difference of opinion, however, as regards the question of the mechanism of this evolutionary process. Three independent theories of the mechanism (R. A. Fisher, 1928; J. B. S. Haldane, 1930; Plunkett, 1933, and Muller, 1932) have been proposed. Wright (1934), although having a different viewpoint on dominance, nevertheless admits the possibility of the

hypothesis of Plunkett and Muller. This is not the place to discuss these theories. Moreover, it really does not matter, for the considerations which follow, which theory of evolution of dominance we accept for our hypothesis.

It need only be emphasized here that the fact that wild genes generally dominate over their mutant allelomorphs is the basis of *all* these theories. It was just this fact which led Fisher to the conclusion that wild genes *become* dominant by selection. But, *mutatis mutandis*, the same reasoning enables us to understand the peculiarities of the pathological genes described above. If the dominance of wild, normal allelomorphs brings us to conceive of the evolution of dominance, then naturally incomplete dominance may be presumed to be the result of an incomplete evolutionary process. In other words the pathological mutations of man have not yet completed the development which leads to absolute recessiveness. Therefore, a considerable majority of genes in man are *as yet not recessive*. However, *they are no longer strictly dominant*, because, as was demonstrated above, they are extremely variable phenotypically (in respect to penetrance). But genes which are sometimes expressed and sometimes not expressed in the heterozygote (**Aa**) should, as a matter of fact, be regarded as recessive in the cases of non-expression and as dominant in the cases of expression, or, better still, as "conditionally dominant". In other words, a considerable majority of genes in man have not as yet become fully recessive, but have already lost a large part of their pathological effect in the heterozygous condition. And from the point of view of evolution of dominance it is natural to suppose that this evolution in man should not yet have completed its course.

Can we propose a hypothesis which would explain this retardation of the evolution of dominance in man? I think that we can. It should be recalled, in this connexion, that natural selection, as a factor influencing the evolution of man, has become a great deal less significant than it was in his savage ancestry, being slowly replaced by new factors—social forces. Natural selection has played a progressively decreasing rôle since man's ancestors first began to use tools even of the most rudimentary kind in work activity. The rôle of natural selection decreases in proportion as man is farther removed from his ape-like ancestors. We may assume, therefore, that the process of evolution of dominance has been retarded along with this general retardation. For while the evolution of dominance is based upon the fact that the mutant gene reduces the vitality of its carrier, it is also true that the use of

implements of labour (and later the development of medicine) fully or partly compensates for this deficiency, and the individual who was formerly mercilessly destroyed by natural selection is by this means enabled to live without impediment and to reproduce. With increased development in technique and industry, with further advancement in medicine, the greater may be the defects (results of pathological mutations) which *Homo sapiens* is able to bear. Since the basic stimulus for the evolution of dominance or for the conversion of mutant genes into recessives has thus disappeared, we come to the conclusion that the recessive pathological genes under present observation arose very long ago, and have in consequence already had time to lose their expression in the heterozygote. Those genes which, on the contrary, have arisen comparatively recently,¹ have not as yet passed through a corresponding period of development and consequently have preserved some degree of dominance.

H. J. Muller, after acquainting himself with the facts presented above and with their interpretation, has proposed to me a further possibility, with which I fully agree. His idea is that the retardation of natural selection in human society could have another result, namely, loss of recessiveness (because of mutations acting oppositely to those which cause dominance) in some of those genes which previously had time to acquire it. Should a loss like this accidentally affect a wild species, the process of redevelopment of the recessiveness of the mutant gene would immediately begin again. But as a consequence of the decline of natural selection in man, the latter can fully stand such a loss, and the loss of dominance would eventually become established. Thus, not only the development of dominance but also its maintenance depends upon natural selection not being relaxed.

The hypothesis presented by the author throws some light on the later ontogenetic expression of "conditionally dominant" genes, mentioned above, as compared to recessive genes. The possibility is not excluded that dominance, at least in individual cases, is conditioned by age, in such wise that the expression of the mutant gene is postponed till the end of the reproductive period. As was stated above, a number of "conditionally dominant" genes are expressed at 30-40 years of age and later. These genes may thus be interpreted as having already become recessive for the reproductive period. And yet they preserve their patho-

¹ It is understood that the expressions "long ago" and "recently" refer chiefly to the period of the origin of man and to the very early period of the history of the species *Homo sapiens*.

logical effect (in the heterozygote) for later life. Hence, it may be supposed that many diseases characteristic of old age are the expression of dominant or conditionally dominant genes which have already become recessive in early life.

The genetics of diseases of old age, which has hitherto been very superficially investigated, acquires special interest in the light of what has been said.

It is worth while to discuss another point in connexion with the question of the age when the mutant gene attains expression. As a matter of fact, one can really regard the tendency of conditionally dominant genes to be expressed at a later age than recessive genes as a particular case of a more general rule which is frequently mentioned in human genetics, namely, that recessive diseases in general are more serious than dominant diseases. However, on the basis of the above a more careful analysis may lead to the very opposite conclusion. This follows, not from general comparisons of recessive with dominant diseases, but from separate comparisons of heterozygous dominants with heterozygous recessives, and of the homozygous dominants with homozygous recessives. Thus, genes express themselves in immeasurably milder form in the heterozygotes in the case of recessive diseases (where they practically do not express themselves at all) than in the case of conditionally dominant diseases. This may be explained by the fact that the recessive gene has completed its evolution, and has lost its pathological effect, while the dominant gene has still to pass through this stage of its evolution. There are grounds for supposing that the homozygote also shows a milder effect in the case of recessives as compared to dominants. For, as was pointed out above, one can presume that at least part of the conditionally dominant genes are lethal in the homozygote (that is, they cause a more serious form than the most serious known recessive diseases). Secondly, as will be noted later, it is possible that some of the recessives also are no longer expressed pathologically even in the homozygote, since their effect also has probably been lost, due to the same evolutionary process. Such characteristics naturally cannot be ascribed to the dominants, since even their heterozygotes have as yet far from completed their evolution.

Thus when we come to the general conclusion that known recessive genes in man tend to a milder (less harmful) phenotypic expression than dominant genes, this in turn requires an explanation. This explanation may easily be formulated as follows: *a gene has a milder effect not because it is recessive but it is recessive because, either from the moment of its origin,*

or by reason of more selection, it gave a milder effect. This is in accordance with the view expressed by Fisher, that the genes with less pathological effect naturally reach the limits of their evolutionary development sooner, lose their harmful effect in the phenotype more quickly, give way to the influence of gene modifiers more easily, than do genes with greater pathological effect.

The latter conclusion is important for two reasons. First, it affords another interpretation for the fact that the penetrance of conditionally dominant genes occurs later than that of recessive genes. Secondly, while the view that recessive genes tend to express more serious cases of disease than dominant genes is purely empirical and has no explanation, the hypothesis formulated by the author has the advantage of yielding to theoretical interpretation.

Classification of genes in man

On the basis of the data presented above, the author proposes the following classification of genes in man, subdivided with respect to dominance.

- (1) Complete dominants. Evidently there are very few of these.
- (2) Conditional dominants, the majority of which show rather poor penetrance. Most mutant pathological genes in man belong to this group.
- (3) Intermediates. This designation should not be taken in the strict sense of the word, that is, in the sense that the phenotypic expression of the heterozygote is exactly intermediate between the expressions of two homozygotes. In practice its expression is closer to one of the latter.
- (4) Recessives. Emphasis should be placed upon the fact that the study of recessive genes more and more reveals a slight expression in the heterozygote. Whether this is the result of an as yet incomplete evolutionary process, or whether this process can be regarded as complete in the sense that the heterozygote has become a wild type, to the degree necessary for the successful survival of the gene carrier, is a question the solution of which is hardly probable in a general form, and which requires a detailed study in every individual case.

Quantitatively, categories (3) and (4), taken together, form the second group of mutant genes in man.

(4 a) Some of the recessives are not always expressed even in the homozygote. There is reason to believe that this is true, for example, for epilepsy, schizophrenia, etc. According to Fisher these may be interpreted as follows. After rendering the heterozygote like or almost like the dominant homozygote the evolution of dominance continues with

the accumulation of modifiers of the homozygote, thus finally inhibiting its expression. However, it must be admitted that the study of this problem is as yet extremely inadequate. We still have very few data which allow us to speak convincingly about the degree to which recessive genes are expressed in the homozygote. There are theoretical and practical reasons for the accumulation of these data, both for deciding the question of whether recessive genes always remain unexpressed in the heterozygote, and of whether they are always expressed in the homozygote.

(5) Indeterminate genes. These comprise only sex-linked genes which are wholly limited to the male sex. It is impossible to distinguish dominants from recessives in this case.

CONCLUSIONS

1. The majority of pathological genes in man:

(a) are conditionally dominant, showing a definite expression in the heterozygote, and

(b) have poor penetrance. This is true both for autosomal as well as for sex-linked genes.

2. Conditionally dominant genes (in the heterozygote) tend to be expressed later in ontogeny than recessive genes (in the homozygote).

3. An explanation is given for these phenomena from the point of view of the evolution of dominance.

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POLLEN GERMINATION IN *BRASSICA CHINENSIS* × *RAPHANUS SATIVUS* F_1 HYBRIDS

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IN 1934 plants of *Brassica chinensis* (Chinese cabbage) were pollinated by *Raphanus sativus* (radish). A few seeds were obtained and five hybrid seedlings were raised in 1935—Nos. 1, 2 and 3 from the family *Brassica chinensis* 45 × Radish 39 and Nos. 4 and 5 from *B. chinensis* 44 × Radish 39. It was obvious from vegetative characters, and later from flowers and fruit, that the plants were intermediate between the parents; and chromosome counts confirmed that they were diploid hybrids with 19 somatic chromosomes, that is 10 from *B. chinensis* plus 9 from radish.

At the end of the heterotype division, besides tetrads, a considerable number of dyads was formed. These indicated the occurrence of restitution nuclei and the formation of pollen grains with the unreduced number of chromosomes. This gave the opportunity of studying the behaviour of diploid pollen on diploid styles, and pollination was carried out between the hybrid plants.

Usually pairs of flowers were pollinated on the day of opening. About 24 hours later the pistils were removed and fixed in a mixture of 70 per cent alcohol (100 c.c.) with formalin (7 c.c. of 40 per cent). Longitudinal free-hand sections were cut, stained by warming in lacto-phenol cotton blue, and mounted in lacto-phenol. The pollen grains on the stigma were observed and the numbers of ungerminated and germinated grains counted. The rest of the section—style, and septum in the ovary—also was searched for pollen tubes, but if germination had proceeded so far that tubes were in the ovary their pollen grains were emptied, and thus unstained, and could be counted on the stigmatic papillae amongst the stained unemptied grains.

A few observations were made on fresh pollen. One anther was taken from each of three opening buds and the pollen mixed in a drop of acetocarmine. The counts of large full grains and empty smaller ones (of varied size) gave these results:

Date	Plant No.	Full	Empty	Full grains %
22. vi. 35	1	17	230	6.9
	2	236	598	39.4
25. vi. 35	3	87	606	14.4
	3	144	255	36.2
9. vii. 35	4	12	309	3.0
3. vii. 35	5	110	627	17.5

Thus there was considerable variation from day to day and probably from plant to plant. This also appears from the figures quoted below for actual observations on the stigmas.

Fixation No.	Pollination		Plant No.		Large full	Small and empty	Large full %	Large germinated %	Emptied %
	Date	Hours	♀	♂					
$F_1 \times F_1$									
207	22. vi	24	1	1	9	62	13	? 11	0
					4	13	24	0	0
					6	32	19	? 50	0
208	22. vi	24	1	2	41	43	49	51	0
					35	37	49	40	0
					93	105	47	39	? 3 No tubes
259	6. viii	25	1	3	18	115	13.5	? 28	0
					5	20	20	0	0
206	22. vi	24	2	1	1	21	5	0	0
					7	118	6	0	0
					81	208	28	? 22	0
202	21. vi	24	2	2	41	62	40	63	0
217	28. vi	24½	2	2	20	16	55	50	0
					25	30	45	32	0
205	22. vi	23½	2	2	23	98	19	26	0
					15	35	30	13	0
					28	70	29	18	0
218	28. vi	24½	2	5	5	55	8	0	0
					12	110	10	17	0
264	7. viii	25	2	4	2	15	12	? 50	0
					0	3	0	0	0
214	27. vi	24	5	2	136	146	48	81	10
					36	42	46	80	3
					73	56	56	90	11
213	27. vi	24	5	5	0	34	0	0	0
					4	53	7	0	0
245	3. vii	26½	5	5	20	90	18	15	0
					12	106	10	? 8	0
$F_1 \times \text{other plants}$									
221	28. vi	24½	5	Radish 59	109	—	100	88	11 tubes
					138	—	100	91	11 "
242 a	3. vii	26½	5	Radish 59	264	—	100	76	26 "
					174	—	100	88	29 "
220	28. vi	24½	5	<i>chinensis</i>	216	—	100	93	? 17 "
					313	—	100	96	6 tubes
243	3. vii	26½	5	<i>chinensis</i>	159	—	100	77	53 "
					72	—	100	78	25 "
244	3. vii	26½	5	Swede 44	116	—	100	91	45 "
					214	—	100	80	48 "
219	28. vi	24½	5	Turnip 36	16	41	28	87	15 ? tubes
				× radish 73	2	32	6	100	0
Other species and crosses $\times F_1$									
228	2. vii	26½	Radish 59	5	16	44	27	88	0
					5	49	9	80	0
227	2. vii	26½	Radish 59	2	9	61	13	89	? 22
					4	52	7	75	? 25
225	2. vii	26½	<i>carinata</i> 56	2	4	26	13	50	0
					7	10	41	86	0
226	2. vii	26½	<i>carinata</i> 56	5	28	43	39	82	? 7
					18	174	9	33	0

Fixation No.	Pollination		Plant No.		Large full	Small and empty	Large full %	Large germinated %	Emptied %
	Date	Hours	♀	♂					
Other species and crosses $\times F_1$									
216	27. vi	24½	Swede 44	1	28	42	40	96	37 style
215	27. vi	24½	Swede 44	2	10	7	59	90	11
					132	171	43	77	44 tubes
223	1. vii	24	(carinata × radish) F_1	2	74	73	50	91	48 "
					12	39	24	92	25 "
224	1. vii	24½	(carinata × radish) F_1	5	8	11	42	88	? 12.5
					10	22	31	100	30
229	2. vii	26	(carinata × cabbage) F_1	2	1	24	? 4	0	0
					46	45	50	89	50 tubes
230	2. vii	26	(carinata × cabbage) F_1	5	36	51	41	94	64 "
					44	85	34	55	50 "
231	2. vii	26	(carinata × cabbage) F_1	Self	45	95	32	62	43 "
					36	73	33	92	22 "
					15	55	21	87	40

At the beginning plant 2 appeared to have more abundant pollen in its anthers than the other plants, and from the counts it seems that this was also connected with a higher proportion of full pollen grains. This plant therefore was used more freely for pollination than the others.

When pollen was fairly abundant only one anther was used on each stigma, but if pollen was not set free easily two or more anthers were used.

Percentages of full pollen on different dates

(Extracted from table above)

Plant No.	June				July			August	
	21	22	27	28	1	2	3	6	7
1	—	13, 24, 19, 5, 6, 28	40, 59	—	—	—	—	—	—
2	40	49, 49, 47, 19, 30, 21	48, 46, 56, 43, 50	55, 45	24, 42	13, 7, 13, 41, 50, 41	—	—	—
3	—	—	—	—	—	—	—	13.5 20	—
4	—	—	—	—	—	—	—	—	12.0?
5	—	—	0, 7	8, 10	31, 4	27, 9, 39, 9, 32	18 10	—	—

After pollination within the F_1 family a total of 28 stigmas from 9 combinations—3 selfs and 6 crosses—was examined. On 8 stigmas on which there were very few full grains no full grains germinated. On the others germination varied from 8 to 90 per cent (and the members of each set of two or three agreed reasonably well), but in all except one set the germ tubes were only short and had not entered the papillae, and the grains were still full of protoplasm. On the three stigmas of set 214, plants 5 \times 2, however, 3, 10 and 11 per cent of the germinated

grains were completely emptied of their contents, and a few pollen tubes could be seen in the ovaries. Even the germ tubes of the partially emptied grains looked more normal than those on the other stigmas, on which they were only about as long as the pollen-grain diameter, and many were somewhat twisted.

Thus in general the pollen of these diploid hybrids is not successful on their diploid styles, but occasionally a few grains do germinate better and enter the stigma and style. It might be suggested that perhaps the successful tubes came from pollen grains with approximately the haploid number of chromosomes, but this does not seem to be the only reason, or occasional tubes would have entered other stigmas, as it is likely that the pollen in any one anther would possess a range of chromosome numbers.

In hybrids in which some good pollen is produced it would seem that the failure of these roughly diploid pollen grains to germinate on the diploid stigmas is itself a powerful factor in producing the characteristic sterility of the hybrids, for only occasional pistils would set a few seed even if all were fully pollinated.

Our plants were largely exposed to open pollination. They stood in a group, and bees were frequently observed visiting their flowers. Other species and hybrids of *Brassica* were growing near and may also have contributed pollen. Comparatively few pods were set and most of them did not contain seeds. Rough counts were made on branches picked at random of the number of pods and of the remains of flowers and flower stalks at the end of the season (on 28. viii. 35) when flowering was finished.

Plant	Pods	Flowers	Pods as % of flowers
1	42	310	13.5
2	24	329	7.3
3	24	304	7.9
4	31	320	9.7

If even imperfect pod development is the result of pollen tube growth and perhaps fertilization of at least one ovule, this count would mean that pollen tubes only entered about one stigma in ten as in the experiments described.

In order to obtain further evidence of the viability of their pollen grains and of the suitability of their stigmas for pollen germination, the *chinensis* × radish hybrids were also used as male parents in pollination on other species and hybrids with different chromosome numbers, and were themselves pollinated by their parent species, and also by swede and an F_1 hybrid from turnip × radish.

On Radish 59 pollen from plants 2 and 5 gave a high percentage germination, although the actual numbers were small, but only a few grains in set 227 were doubtfully emptied and only one tube was found nearly as far as the base of a papilla in set 228.

A large proportion of pollen from the same two hybrids also germinated on the stigmas of *B. carinata* 56 ($2n=34$), but only two grains were possibly emptied and no tubes were found in the pistil.

On Swede 44, however, pollen from hybrids 1 and 2 germinated well. About 40 per cent of the germinated grains were emptied of their contents and a number of tubes were found in the stigmas, styles, and ovaries. Later a few more flowers were pollinated and developed small pods in which several ovules had undergone some development, thus indicating again the entrance of the hybrid pollen tubes.

An F_1 hybrid between *B. carinata* \times radish ($2n=17+9=26$) was pollinated by plants 2 and 5. On three out of four stigmas germination was good, some grains were emptied and one tube was seen well down the ovary.

The same two *chinensis* \times radish plants were used also to pollinate an F_1 hybrid between *B. carinata* and *B. oleracea* (cabbage). Many grains germinated and a number of tubes were found in stigma and style and ovary.

Thus from the results on swede and the *carinata* \times radish and *carinata* \times cabbage hybrids it is obvious that most of the pollen grains of the *B. chinensis* \times radish hybrids were capable of germination and the production of vigorous pollen tubes. The failure on styles of their own family will be discussed below.

Several crosses showed that other pollen was able to grow in the styles of the *chinensis* \times radish F_1 hybrids. Plant 5 was pollinated by Radish 59 on two days. On all four stigmas germination was high and many pollen tubes were seen as far as the ovaries. The actual number of pollen grains was much higher than in the reciprocal cross, but even so the difference in pollen tube growth was striking.

Pollination by *B. chinensis* gave a similar result, and a number of tubes were found well down in the ovaries. Swede pollen also germinated freely and the germ tubes were found all the way down into the ovaries.

The failure of the diploid pollen on the stigmas of their own family must then be the result of incompatibility factors or the departure from the usual balance of $1n$ pollen tubes in $2n$ styles.

For several reasons it is not likely that incompatibility factors would be involved to such an extent. Our plants of *B. chinensis* and radish

generally have set some seed from artificial self pollination or from bagged inflorescences, and two different plants of *B. chinensis* were parents of these F_1 hybrids. Pollen from the F_1 turnip \times radish, with the same number of chromosomes but different parent plants, was equally unsuccessful on the F_1 stigmas of *chinensis* \times radish, in spite of the possible identity of self sterility factors in *chinensis* and turnip (or other species of the $n=10$ group). In addition it is now being found at Cambridge that tetraploid hybrids from *R. sativus* \times *B. oleracea* will self quite readily, in spite of the marked self incompatibility of *B. oleracea*.

Normally in radish, pollen grains with 9 chromosomes germinate on, and their germ tubes grow in, styles with 18 chromosomes. Similarly in *B. chinensis* 10-chromosome pollen tubes grow in 20-chromosome styles. In the F_1 hybrids between these species the pollen grains may have from 9 to 19 chromosomes—probably more nearly 19—and they have to germinate on 19-chromosome styles. The balance is disturbed and only a few succeed in entering the papillae and growing. If these few succeed because of low chromosome numbers the theory of Watkins (1932) is fully supported. The progeny, now growing, should then have considerably less than 38 ($=2 \times 19$) somatic chromosomes.

It seems more likely to be the result of ordinary fluctuations—genetic, physiological, or environmental—that produce exceptions to the rule, and some evidence in support of this is appearing from the behaviour of pure species pollen in some other *Brassica* crosses.

With radish ($2n=18$) as female parent and *chinensis* \times radish hybrid pollen the balance also was unusual, and growth not successful, but with radish pollen ($n=9$) and *chinensis* pollen ($n=10$) on the hybrid stigmas ($2n=19$) the normal relationship was more closely reached, and more pollen tubes entered the stigma, as would be expected on Watkins' view.

When other species are crossed presumably other factors are introduced, and with swede ($2n=36$) pollination was partially successful in both directions. The hybrid pollen did not do well on *B. carinata* styles ($2n=34$), but the pollen tubes were able to grow on F_1 stigmas of *B. carinata* \times *B. oleracea* ($2n=26$) and of *B. carinata* \times radish ($2n=26$), so that when species other than the parents of the hybrid are involved the relation between the chromosome numbers of pollen tubes and styles may be upset by other factors.

The interest of these results is that this sterile F_1 produces a large amount of diploid pollen, which is normally functional in several crosses, but is unable to grow except rarely on the stigmas of the diploid F_1 itself. In any genus in which this effect occurs the artificial or natural produc-

tion of polyploids may clearly be restricted. The possibility is not unexpected since Watkins has already pointed out (1932) that it does occur in some genera that diploid pollen, as a result of the disturbance of the normal relation between pollen tube and style, may fail to function on diploids, although it behaves normally on tetraploids. The correctness of this explanation is probably confirmed by the results of Terasawa, who has obtained constant amphidiploid hybrids of this cross, *B. chinensis* \times *R. sativus* ($2n=38$). His F_1 plants (1932) gave only a few seeds, and thus resembled ours. The amphidiploid F_4 plants were highly fruitful from selfing and gave many seeds: in one experiment (1933) 128 flowers gave 102 seeds. This certainly appears to show that the diploid hybrid pollen did grow more successfully on the pistils of the amphidiploids than on those of the diploids, in accordance with the expectations of Watkins.

SUMMARY

Diploid pollen, with 19 chromosomes, from the F_1 hybrid plants ($2n=19$) of *B. chinensis* \times *R. sativus* usually failed to grow on the F_1 stigmas. It was, however, fully capable of germination on the stigmas of swede ($2n=36$) and of some other crosses. Also pollen of radish, *B. chinensis*, and swede was able to grow on the F_1 stigmas.

The sterility of the F_1 plants is attributed to the alteration of the chromosome balance between pollen and style from the usual $1n:2n$ to $2n:2n$.

The work described was carried out at the Plant Breeding Institute, School of Agriculture, Cambridge.

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STUDIES OF MULTIPLE ALLELOMORPHIC SERIES IN THE HOUSE-MOUSE¹

I. DESCRIPTION OF AGOUTI AND ALBINO SERIES OF ALLELOMORPHS

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THE discovery of series of multiple allelomorphs in a large number of animals and plants has raised questions of considerable interest regarding the manner in which the genes influence development. The evidence shows in many cases that these series have arisen through repeated mutations at the same locus in the chromosome, and that the members of such a series represent different alternative conditions of the gene. The phenotypic effects of the allelomorphs of the same series have been found to be frequently similar in kind, affecting the same characters in similar ways, and these effects often appear to differ from each other chiefly in degree or quantity. The evidence for these statements has been collated and critically reviewed by Stern (1930), who has also called attention to certain significant exceptions to the simple rule of qualitatively similar effects of multiple allelomorphs.

As part of a general physiological theory of heredity, Goldschmidt (1927, and previous papers) has assumed that, since the effects of allelomorphs differ in general only quantitatively, these effects are due to quantitative variations in the gene, the effect of a gene on development being in proportion to its quantity. The latter and more general assumption is based in part on the proportionality which exists in many cases between the numbers of one allelomorph present in the individual and the quantity of effect produced. The assumption that genes vary quantitatively cannot be tested directly by evidence from phenotypic effects; but the special case which should follow from the general principle, namely, that the *effects* of changes in the same gene bear a purely quantitative relation to each other, is a question of fact and is susceptible of direct test.

Critical evidence on the phenotypic effects of multiple allelomorphs should of course include quantitative measurements of the characters

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affected, and these are generally difficult to obtain. A second requirement is that the individuals containing different members of the allelomorphic series should be comparable in respect to other genes and to other conditions.

The results of the few analyses in which these requirements have been at least partially met are not entirely conclusive in respect to the question at issue. Wright's (1925) measurements of the intensities of black and yellow pigment in the hairs of guinea-pigs with different combinations of the albino series of allelomorphs showed a general reduction from the intense colour of the wild type (**C**) through intermediate stages, brought about by combinations of three intermediate allelomorphs (**c^k**, **c^d**, **c^r**), to the nearly white coloration of the albino (**c^a**). The order of effect of these genes was slightly different for black and for yellow. Wright assumed that the genes of this series determined different rates of one process fundamental to pigment production, the irregularities in the order of effect on different pigments being due to threshold differences brought about by other genes. The gross effects of the genes appeared to differ quantitatively, but the nature of the changes in the pigments could not be described by the methods used.

Dobzhansky (1930) compared the effects of three allelomorphs in *Drosophila melanogaster* (stubble, stubbloid and wild type) on bristle number and length, wing length and leg length. The order of quantitative effect of the three allelomorphs on these different structures was found not to be the same, from which Dobzhansky concluded that the effects of the allelomorphs do not differ only quantitatively. Goldschmidt (1932), however, has pointed out that such differences in order of effect do not constitute evidence against the quantitative hypothesis, since they may be consequences of differences in the rate and order of differentiation of the different characters affected, processes which may occur independently of the allelomorphs in question.

The effects on eye size of the Bar allelomorphs in *D. melanogaster* have been analysed by Sturtevant (1928). Although the effects of each allelomorph bear a quantitative relation to the numbers of that allelomorph present (e.g. 0 to 4 Bar genes), one allelomorph (Bar) is fully dominant to another (Infrabar) in all combinations in which the two allelomorphs have been found, as has been pointed out by Wright (1929). Since in combinations with infrabar, one, two or three bar genes give the same quantitative effects on eye size, it seems impossible to conclude that the relations between the effects of these genes are purely quantitative. Whether relations of "allelomorphs" at the bar locus may be regarded

as typical of allelomorphs in general is doubtful in view of the unequal crossing-over known to occur in this region.

Other cases have been discussed by Stern, who has concluded that the evidence at present does not permit of a decision between the alternative hypotheses of qualitative and purely quantitative differences in the effects of multiple allelomorphs.

It should, however, be possible to throw some additional light on this question by comparing the actual products affected by the allelomorphs. If these are found to differ only in quantity in the different compounds of an allelomorphic series, the assumption of qualitative differences is unnecessary. If differences in kind also appear, then the need for further analysis is indicated, which must determine whether such differences could have arisen by quantitative variations in other preceding products or reactions.

As materials for studying the nature of the relationships between the effects of allelomorphs, two series of multiple allelomorphs in mice affecting the melanin pigments of the hair seemed favourable. In studying such colour effects there is at once the possibility of making objective measurements of the quantity and of the quality of pigment present, and of eventually making direct observations of some of the chemical steps by which pigments are produced, since the processes involved in the formation of melanin are known in outline.

THE AGOUTI SERIES

Both series of allelomorphs have been known for several years, although neither has been thoroughly described (cf. Dunn, 1928 and Keeler, 1931). One of these, known as the "Agouti" series, affects chiefly the relative distribution of black (or brown) and yellow pigments over the body and in the individual hairs. It consists of the following members (the effects on wild type only are described):

- A^Y**: all hairs yellow with occasional dark granules. Dominant to all other members of the series and lethal when homozygous. Shows tendency to become fat and sterile with age and acts as a modifier of white spotting, these effects not being shared by other members of the series.
- A^L**: agouti with light belly. Hairs on back with narrow yellow band near the tip. Belly hairs light yellow for most of their length. Sometimes referred to as agouti white belly, **A^w**, but known now not to affect intensity of yellow. Dominant to **A**, **a^t** and **a**.

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A or + : agouti with grey belly, the wild type of *Mus musculus*. Hairs on both back and belly black with yellow band near tip. Dominant to **a**; compound **Aa^t** is agouti with yellow belly.

a^t: black-and-tan; back black; belly yellow with dark patch on throat. Dominant to **a** (Dunn, 1928).

a: non-agouti, black.

Although extensive evidence shows that these genes segregate as allelomorphs, their relations to each other are in some respects unusual. The peculiar effects of **A^t** on pigment, viability and spotting suggest that this at least may not be an allelomorph but possibly a deficiency so near to the agouti locus that it shows no crossing-over with it. **a^t** seems to bear the same relation to black which **A^L** bears to wild-type agouti, that is each allelomorph changes the belly colour to yellow or lighter shade, and it has been suggested by Pincus (1930) that a mutation closely linked to the agouti locus has occurred in an agouti stock resulting in light-bellied agouti and in a black stock resulting in light-bellied black (black-and-tan). No evidence for this exists, since crossing-over between the assumed light-belly mutation and the agouti locus has not been observed.

For the objects we have had in mind, the agouti series does not provide the most favourable material, and we have used it chiefly to provide different backgrounds against which to observe the action of another series of allelomorphs. For present purposes it is immaterial whether the agouti members are "good" allelomorphs or not. We shall present later some evidence on the quantities of pigment found in various members and shall then discuss this series in more detail.

THE ALBINO SERIES

A second series of four allelomorphs is known in mice, affecting the intensities of both black and yellow pigments in the hairs. It consists of the following members (the effects described are those of homozygotes on wild-type agouti):

C or + : wild type—full intensity of black, brown and yellow. Completely dominant to other members. Eyes dark.

c^{ch}: ruby or chinchilla dilution. Sometimes referred to as **c^r**.¹ Black reduced to dull black or very dark slate; yellow reduced to

¹ The original symbols **c^r** and **c^d** have been replaced here by **c^{ch}** and **c^h** in order to avoid confusion with albino allelomorphs **c^r** and **c^d** in the guinea-pig (Wright, 1925), which were discovered earlier and in which **c^r** represents the lighter condition.

light yellow or cream. Eyes dark. First described by Feldman (1922).

- c^h : Himalayan dilution. Sometimes called extreme dilution c^d . Black reduced to pale brownish grey; yellow reduced to white. Eyes dark. First described by Detlefsen (1921).
- c^a : complete albinism; no pigment in either hair or eyes.

The experiments of Detlefsen and of Feldman indicated that the dilute mutant forms (c^{ch} and c^h) were probably allelomorphs of the long-known albino mutation (c^a). Each was completely recessive to the normal condition (C) and formed compounds intermediate in colour when crossed with c^a and with each other. The dominance order appeared to be $C \gg c^{ch} > c^h > c^a$.¹ Extensive experiments were not made to test this relationship and to exclude the possibility that the differences in density of pigment found might be due to other genes (modifiers). The effects of these genes in all the compounds and in combinations with other genes have not been described previously.

In preparing material for the present study it was thus necessary (1) to confirm the fact of allelomorphism of the four factors involved, (2) to study the effects of these on coat colour in various combinations, and (3) to prepare the combinations on as nearly comparable genetic backgrounds as possible, so that the effects of genes outside of the albino series might be minimized. Combinations of C , c^{ch} , c^h , and c^a in homozygous condition, and in the compounds $c^{ch}c^h$, $c^{ch}c^a$, $c^h c^a$, a total of seven genotypes, were made with the five agouti allelomorphs, other colour genes being of the wild type (B , black; D , dense; P , dark eyes, etc.). This yielded thirty-five combinations, in which the effects of the albino allelomorphs could be observed on backgrounds varying in pattern from yellow to black. The gene brown (b) was then substituted for black (B) in the seven albino combinations with A^I and a of the agouti series, which yielded fourteen combinations in which the effects of the albino allelomorphs on brown and yellow could be observed. A few combinations of c^{ch} and c^h with other colour-dilution genes (d , maltese dilution and p , pink-eyed dilution) have been made, but all of the possible combinations have not yet been prepared.

SOURCES OF MATERIAL

The c^{ch} gene was obtained from two white-bellied agouti animals kindly supplied by Prof. W. E. Castle in June 1928. These were descendants of the original c^{ch} animals described by Dr Feldman and con-

¹ \gg indicates complete dominance, $>$ indicates incomplete dominance.

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formed to his description. They proved to be $A^L A^L B B c^{ch} c^{ch}$. These were crossed with inbred stocks of black-and-tan, yellow, agouti, and black obtained in 1927 from English fanciers and maintained in our laboratory.

The c^h gene was obtained from three extreme dilute animals kindly supplied by Dr H. W. Feldman in March 1930. These proved to be $A A b b c^h c^h$ and gave rise to a line of this genotype which was inbred for two years and was crossed with other stocks in preparing the various combinations with c^h .

The c^a gene was obtained from albinos which appeared in a stock of English blacks. In making combinations involving brown, the Bagg strain of albinos was used, since this is known to be $A A b b c^a c^a$.

Thus a number of different stocks were used in making the first combinations, and these may have differed in minor modifying factors affecting colour intensity, although the results show that the effects of such other genes, except in two cases, were relatively unimportant. The relations between the albino allelomorphs were studied chiefly in combinations with A^L and were extracted after successive back-crosses to an inbred stock of $A^L A^L B B C C$ (our line 101 yellow-bellied agouti), which constitutes also the chief hereditary background of the other combinations. This standard stock has been brother-sister inbred in our laboratory for fifteen generations and has shown no hereditary variation in depth of colour.

From the breeding experiments required to produce these combinations were obtained data confirming the allelomorphism of C , c^{ch} , c^h , and c^a , and a preliminary gross description of the effects of these genes on yellow, black and brown pigments.

ALLELOMORPHISM OF THE c GENES

The ratios resulting from crosses involving members of this series need not be quoted in detail, since no exceptions to the expectations based on the assumption of allelomorphism have been found.

Crosses of CC animals (with B or b , combined with A^Y , A^L , A , a^t or a) with $c^{ch} c^{ch}$, $c^h c^h$ and $c^a c^a$, containing any combination of these other genes, show complete dominance of C in F_1 and regular monohybrid ratios in F_2 and back-crosses. No effects of c^{ch} , c^h or c^a on viability have been noted. Crosses of $c^{ch} c^{ch}$ animals containing the genes for black (B) in combination with A^L , A , a^t or a , with $c^h c^h$ animals containing these combinations produce in F_1 compounds $c^{ch} c^h$ which are intermediate in colour between the $c^{ch} c^{ch}$ and $c^h c^h$ parents,

and in F_2 segregation occurs in the ratio of $1/4$ $c^{ch}c^{ch}$; $1/2$ $c^{ch}c^h$; $1/4$ c^hc^h . In agoutis, A^L or A , the separation of these three types is easily made. The $c^{ch}c^{ch}$ agoutis are somewhat lighter than type, the bases of the hairs being dull black and the tips light cream colour; in $c^{ch}c^h$ the bases of the hairs are medium brown, the tips white; while in c^hc^h , black is reduced to very light brown or tan, and yellow is reduced to white. In black-and-tans ($a^t a^t BB$) separation is also clear. C is shiny black with yellow belly, $c^{ch}c^{ch}$ is dull black with white or nearly white belly; $c^{ch}c^h$ is medium brown with white belly; and c^hc^h is very light brown or tawny with white belly. In blacks (aaB) separation of $c^{ch}c^{ch}$ from C is sometimes difficult, although with practice $c^{ch}c^{ch}$ may be distinguished by the lesser saturation of black which tends to be dull and slate coloured near the base of the fur, and especially by the shade of the hairs lining the ears which in C forms are yellowish, in $c^{ch}c^{ch}$ creamy or nearly white. $c^{ch}c^h$ mice with aaB are brown, c^hc^h black, much like the chocolate coloration of abC mice.

Crosses of $c^{ch}c^{ch}$ animals containing also the gene B in combination with A^L , A , a^t or a with albinos produce in F_1 compounds $c^{ch}c^a$, which are identical in appearance with the $c^{ch}c^h$ animals described above, and in F_2 segregation of $c^{ch}c^{ch}$, $c^{ch}c^a$ and c^ac^a is readily discernible in a $1 : 2 : 1$ ratio. In none of the combinations examined have we been able to find any constant differences in appearance between $c^{ch}c^h$ and $c^{ch}c^a$ animals. As shown in Part III, c^h and c^a have about the same effect in reducing the total quantity of pigment when combined with c^h .

Crosses of c^hc^h animals containing also B together with A^L , A , a^t or a with albinos produce in F_1 compounds c^hc^a which are nearly white, but always show some traces of pigment giving them a slight dusky tone. In F_2 three classes appear as $1/4$ c^hc^h , $1/2$ c^hc^a , $1/4$ c^ac^a . The two latter classes can always be separated by eye colour, c^hc^a having dark eyes and dark pigment ring at birth, c^ac^a having pink eyes and no pigment ring. c^hc^h and c^hc^a are usually easily separable by the greater darkness of c^hc^h , but in some combinations the two may overlap and progeny tests must be made.

These results show that C , c^{ch} , c^h and c behave as allelomorphs, only two of these genes ever being present in any individual and only one in any gamete. No reversion to full colour occurs after crosses between c^{ch} , c^h or c^a forms in any combination. The dominance order of effect on coat colour in combinations with B and either A^L , A , a^t or a is $C \gg c^{ch} > c^h > c^a$.

COMBINATIONS OF THE **c** SERIES WITH BROWN

When the members of the **c** series are combined with brown (**b**) instead of black (**B**), their relative effects on coat colour are somewhat different. As in the case of the black combinations, **C** appears to be completely dominant to c^{ch} , c^h and c^a . In **A^Lb**, and **Ab** combinations, $c^{ch}c^{ch}$ is distinguished from **C** only by the lighter shade of the yellow band on the back hairs and by the lighter shade of the yellow belly hairs. In these parts **C** is yellow, $c^{ch}c^{ch}$ is cream. The brown parts of the hair are alike in **C** and $c^{ch}c^{ch}$. The separation of **Abc^{ch}c^{ch}** from **AbC** cannot always be made with certainty. Dilute brown agoutis (**A^Lbbc^hc^h** and **Abbc^hc^h**) are, however, easily distinguished from brown agoutis (**A^L** or **AbbC**). The brown pigment in $c^h c^h$ forms is reduced to light brown or tan; and the yellow parts appear white.

Chinchilla dilute brown-and-tan animals (**a^ta^tbbc^{ch}c^{ch}**) resemble full-coloured brown-and-tans (**a^ta^tbbC**) in the colour of the back, which is full brown or chocolate in both forms. In the **C** forms, however, the belly colour is yellow; in $c^{ch}c^{ch}$ it is light cream colour or nearly white. Extreme dilute brown-and-tans (**a^ta^tbbc^hc^h**) are very light brown dorsally and clear white ventrally.

In combination with non-agouti (**a**) and brown (**b**) the genotypes **C**, $c^{ch}c^{ch}$ and $c^h c^h$ are indistinguishable. c^{ch} even in compound with c^h does not visibly dilute brown. $c^{ch}c^a$ is slightly lighter brown than **C** but cannot always be distinguished from it; $c^h c^h$ is definitely lighter than **C**, of about the shade of $c^h c^h$ black. In all brown combinations $c^h c^a$ is generally white with brown eyes, so that as far as coat colour is concerned c^a acts as a dominant to c^h . However, we found $c^h c^a$ browns from one family which did develop some light brown pigment in the coat, in a few cases almost as much as appears in $c^h c^h$ browns. From the $c^h c^a$ browns, dark $c^h c^h$ browns were isolated and inbred and showed a monohybrid difference from the normal light $c^h c^h$ browns. There is thus at least one other gene independent of the **c** series which affects the intensity of pigment. This gene is being studied further. Leaving aside the effects of such genes, the dominance order of the **c** genes in their effect on brown is probably about as follows:

$$C = c^{ch} \gg c^h = \text{or} > c^a.$$

COMBINATIONS OF THE **c** SERIES WITH YELLOW **A^Y**

All combinations of the **c** genes with yellow (**A^Y**) have not yet been obtained on comparable genetic backgrounds, due to the difficulty of standardizing the darkening genes which exist in many strains of mice,

and when introduced into yellow produce "sooty" yellow, sable, and similar variations in darkness. Preliminary results from experiments in which combinations of A^Y , B and the c genes are being extracted from an inbred agouti stock in which such modifying genes have been standardized show (1) that C is fully dominant to c^{ch} , c^h and c^a ; (2) that c^{ch} has a considerable effect on yellow, reducing it to a pale but still clear yellow shade in $c^{ch}c^{ch}$; (3) that c^h entirely prevents the appearance of yellow, $A^Yc^hc^h$ being clear white. The compound $A^Yc^{ch}c^a$ appears to be white as is also c^hc^a . $c^{ch}c^h$ has not yet been obtained.

The order of effect on yellow is probably

$$C \gg c^{ch} > c^h = c^a.$$

COMBINATIONS OF c SERIES WITH p

A few combinations of other colour genes with those of the c series have been obtained but not extensively studied as yet. The gene p (pink eye) is known to have a considerable diluting effect on black pigment, somewhat less effect on brown, and apparently none at all on yellow. $AppBC$ (pink-eyed black agouti) is superficially yellow, but the bases of the hairs are light slate colour. $AppBc^{ch}c^{ch}$ is creamy buff colour, much lighter than $APBc^{ch}c^{ch}$, due apparently to the cumulative diluting effect of c^{ch} and p on black and the diluting effect of c^{ch} alone on yellow. In the present stock of $AppBc^{ch}c^{ch}$, the yellow appears to be somewhat more intense than the yellow in $A^YPBc^{ch}c^{ch}$. $AppBc^{ch}c^a$ also shows traces of yellow pigment of a light cream shade, and in $APBc^{ch}c^a$ yellow is also reduced to cream. A^L or A with $ppBc^{ch}c^h$, $aappBc^{ch}c^h$, $aappbbc^{ch}c^h$ and the c^hc^a compounds of these types are all clear white with pink eyes indistinguishable from full albinos. Since the dark-eyed (P) forms of these genotypes develop considerable dark pigment (black or brown) it is apparent that c^h and p have cumulative effects on the dilution of these pigments. c^h appears to inhibit yellow entirely both in combination with P and with pp . From these as yet incomplete observations the order of effect of the c genes when combined with p appears to be:

$$(1) \text{ on black } C \gg c^{ch} > c^h = c^a.$$

$$(2) \text{ on yellow } C \gg c^{ch} > c^h = c^a.$$

COMBINATIONS WITH d

The gene d (maltese or blue dilution) reduces black to blue-grey and yellow to cream. One combination of this gene with c^{ch} has been made up, $A^LBddc^{ch}c^{ch}$. This is lighter than $A^LBDc^{ch}c^{ch}$. The black parts

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of the agouti pattern are light blue; the yellow parts are white or nearly so; so that c^{ch} and d appear to have cumulative effects on black and possibly on yellow.

SUMMARY

From these observations the genes of the c series in mice appear to dilute both black and yellow pigments in the hair. Yellow is more affected than black, being markedly diluted by c^{ch} , and entirely suppressed by c^h and c^a . Black appears to be but little affected by c^{ch} , considerably diluted by c^h , and eliminated only by c^a . Brown seems not to be affected at all by c^{ch} , and although diluted by c^h is relatively less affected than black by this gene. A rough description of the relative order of visible effects of these genes on these three colours is given below:

	CC	Cc ^{a1}	c ^{ch} c ^{ch}	c ^{ch} c ^h	c ^{ch} c ^a	c ^h c ^h	c ^h c ^a	c ^a c ^a
Yellow	4	4	2	?	0.5	0	0	0
Black	4	4	3	2	2	1	0.5	0
Brown	4	4	4	4	3	2	0	0

(or 0.5)

$$^1 c^a = c^{ch}, c^h \text{ or } c^a.$$

In this table the density of the pigment in wild type ($C+$) is represented by 4, the apparent relative reduction in density by 3, 2, etc. These are based on gross estimations and not on measurements.

The c genes affect these pigments in the same order, i.e. C , c^{ch} , c^h , c^a ; each effect which is apparent is of the same nature, to reduce the intensity of the pigments.

The effects of these genes are thus essentially similar to those of the albino allelomorphs in the rat and rabbit, and especially in the guinea-pig where they have been intensively studied by Wright (1925, 1927). A more detailed comparison with the other rodent series will be made after the quantitative data on the mouse have been presented.

As one examines the hair colours in the combinations in these several series, the impression is one of a general reduction in *quantity* of pigment from $C \rightarrow c^a$. This appearance raises the question whether all differences brought about by the members of this series of allelomorphs can be adequately described in terms of the quantity of pigment present in each member, or whether mutations at this locus also effect other changes in the pigments.

In order to answer this question it is necessary to *measure* the actual *amounts* of pigment present in different members of the series; and to search for other measurable effects of these genes on pigment. The first

step in such a study was to develop a technique for the extraction, purification and estimation of the amount of melanin pigment which is responsible for the coloration of the hairs. (In the following report only the black and brown pigments will be considered, since we have not yet developed a comparable quantitative technique for measuring the amounts of yellow pigment.) The melanin obtained from each member was then described in two other ways, independently of the amount present. First the form and sizes of the pigment granules in which melanin is organized were measured, and second the characteristics of the melanin from each member were studied in solution. The chief measure found useful in this study was colour density per unit of concentration, as measured by relative light absorption in a colorimeter.

In the following paper of this series these techniques are described in detail, since the methods which we have developed appear to be of general usefulness for quantitative work on pigments. The results obtained to date will be given in a third paper which will also contain a preliminary interpretation of the action of the genes of the *c* series on hair pigments.

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CHROMOSOME NUMBERS IN *CRASSULA*

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(With Twenty-eight Text-figures)

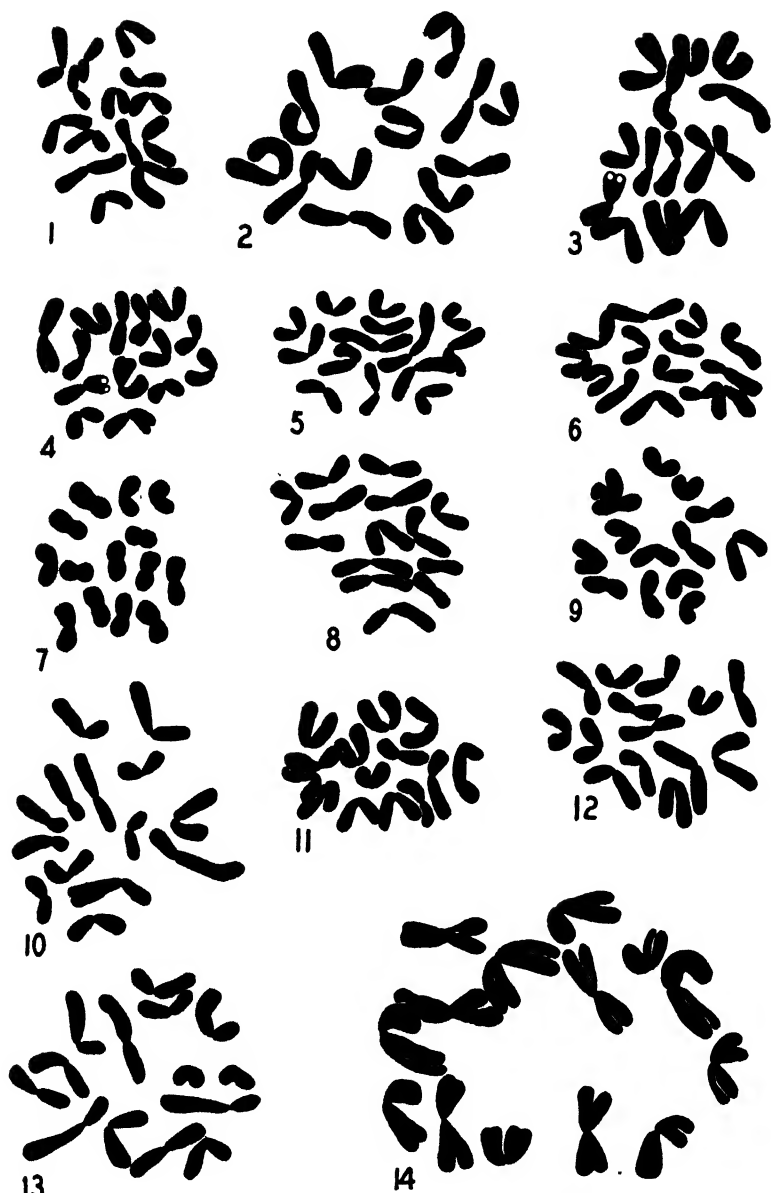
INTRODUCTION

VARIOUS writers have observed that the genera in the Crassulaceae are not well defined. In 1933, as an approach to an understanding of the phylogenetic relationships in the family, a cytological survey of the Crassulaceae was begun. It was planned, in so far as time and material would permit, to analyse, first in a preliminary, and finally in a detailed manner, the chromosomal situation existing in the several genera of the family, and to correlate that knowledge with the available taxonomic treatments of those genera. The purpose of the present paper is to report the introductory study of *Crassula* L.

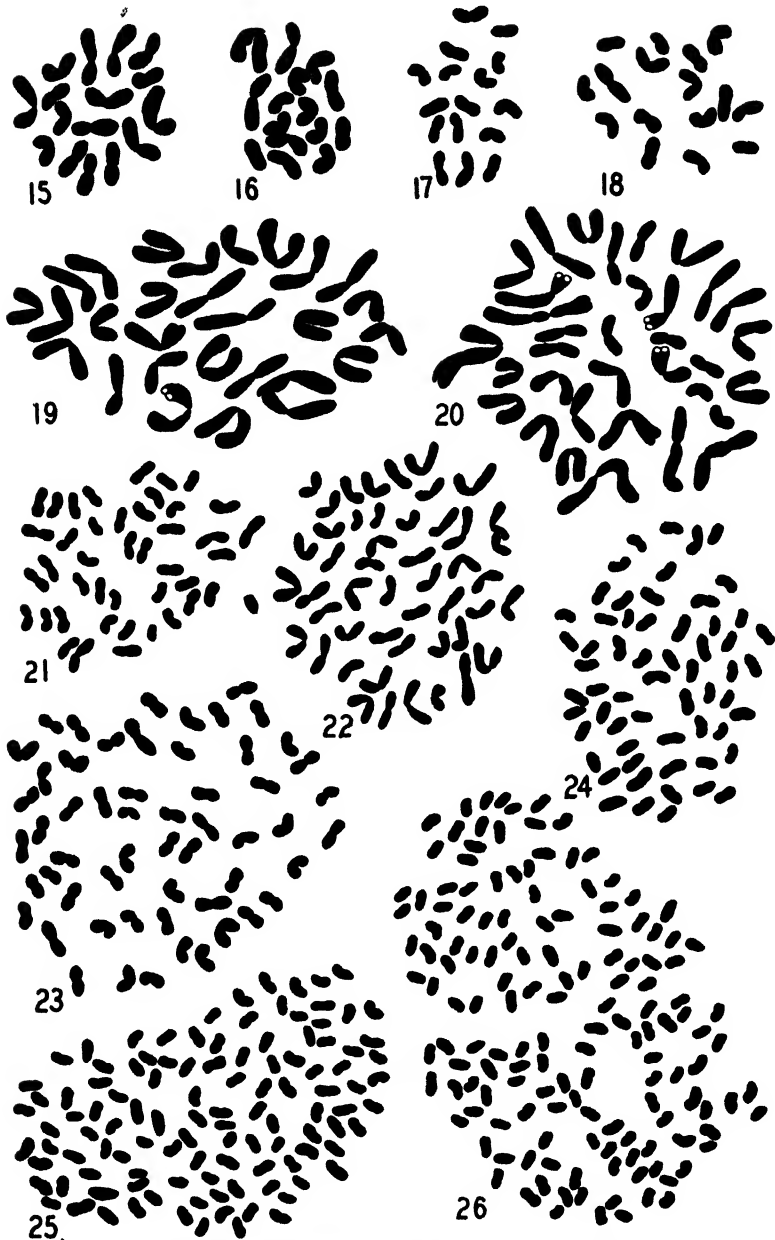
Estimates of the size of the Crassulaceae and of its individual genera vary considerably. According to *Index Kewensis*, *Crassula* is the second genus in size in the family, and *Sedum* is the first. *Crassula* includes, on the basis of that *Index*, 421 recognized species to which 166 others are reduced, 101 of these latter species being reduced from other genera. Similarly, sixty-five species of *Crassula* are reduced extra-generically. These figures afford an indication of the degree of systematic inexactitude that prevails in the demarcation of genera within this family. Berger (1930) reduces to *Crassula* some of the genera recognized in *Index Kewensis*, but he considers *Crassula* to include less than 300 species. About 230 of these species are in South Africa, a number in tropical—chiefly eastern—Africa, one in southern Arabia, several in Madagascar, and the members of section Tillaeoideae distributed throughout the world, but more particularly in the southern hemisphere.

Chromosome numbers for three species of the subfamily Crassuloideae were reported by Skovsted (1934); these species are marked by asterisks in the outline of the plants investigated. Numbers for twenty-seven additional species are given in this report.

The writer expresses appreciation to Dr Orland E. White, Director of the Blandy Experimental Farm, for criticism of this study.



Figs. 1-14. Fig. 1, *Rochea*. Figs. 2-14, *Crassula*. All metaphases except Fig. 14 which is a drawing of prophase. All have $2n=14$. $\times 3260$.

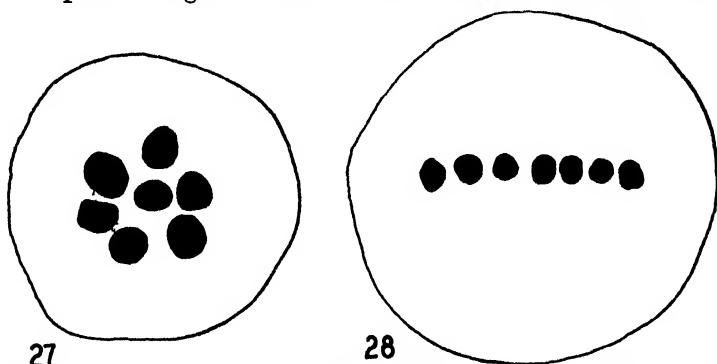


Figs. 15-26. All metaphases of *Crassula* species. Figs. 15-18, $2n=16$; Fig. 19, $2n=28$; Fig. 20, $2n=34$; Fig. 21, $2n=42$; Fig. 22, $2n=48$; Fig. 23, $2n=56$; Fig. 24, $2n=ca. 60$; Fig. 25, $2n=ca. 112$; Fig. 26, $2n=ca. 148$. $\times 3260$.

MATERIALS AND METHODS

Plants of twenty-five species from E. O. Orpet, Santa Barbara, Calif., and gift specimens of *Crassula Peglerae* from Reid Moran, La Canada, Calif., and of *C. arborescens* from the Valley View Greenhouses, Charlottesville, Va., were counted. Only one plant of a species was examined. Specimens from these plants will be placed in the Bailey Hortorium, Cornell University.

Aceto-carminic smears were used; Figs. 1-26 were drawn from smears of root tips and Figs. 27 and 28 from smears of anthers. Prior to



Figs. 27 and 28. Fig. 27, $n=7$, 1st metaphase of pollen mother cell division in *C. hemisphaerica*. Fig. 28, $n=7$, pollen grain division in *C. Peglerae*. $\times 3290$.

smearing, the material was fixed for about 10 min. in Carnoy's fluid. Excellent preparations were obtained. The chromosomes of some of the species are favourable for morphological and structural study. Constrictions were often clearly present. All the drawings were made $\times 4900$. A Zeiss microscope and Zeiss camera lucida were used in conjunction with Wratten filters.

OBSERVATIONS

The species investigated together with their determined numbers are arranged below in accordance with Berger's (1930) monograph of the family:

Crassula L.

	n	Fig.	$2n$	Fig.
Sect. I. Tillaeoideae				
Div. 5. <i>Glomerata</i>				
* <i>C. phanaceoides</i> (Hochst.) Fisch. & Mey.	8	—	—	—
(<i>Tillaea pharnaceoides</i> Hochst.)				
Div. 7. <i>Lycopodioides</i>				
* <i>C. pseudolycopodioides</i> Din. & Schinz.	—	—	16	16
Div. 9. <i>Corallina</i>				
— <i>C. corallina</i> Thunb.	—	—	16	17
<i>Crassula</i> sp., near <i>corallina</i>	—	—	16	18

Sect. II. Stellatae				
Div. 2.	<i>Spatulata</i>	<i>n</i>	Fig.	<i>2n</i> Fig.
	<i>C. spatulata</i> Thunb.	—	—	ca. 148 26
	<i>C. sarmenlosa</i> Harv.	—	—	ca. 60 24
Div. 3.	<i>Lactea</i>			
	<i>C. multicava</i> Lem.	—	—	ca. 112 25
Div. 4.	<i>Arborescens</i>			
	<i>C. arborescens</i> (Mill.) Willd.	—	—	42 21
Div. 5.	<i>Cordata</i>			
	<i>C. cordata</i> Thunb.	—	—	16 15
Sect. III. Tuberosae				
	<i>*C. nemorosa</i> Endl.	44-46		
	(<i>C. nivalis</i> Harv.)	2	—	—
Sect. IV. Campanulatae				
Div. 1.	<i>Acutifolia</i>			
	<i>C. tetragona</i> L.	—	—	48 22
Div. 2.	<i>Perforata</i>			
	<i>C. perforata</i> Thunb.	—	—	14 - 12
Div. 3.	<i>Harveyi</i>			
	<i>C. sarcocaulis</i> Eckl. & Zeyh.	—	—	28 19
Div. 6.	<i>Perfoliata</i>			
	<i>C. falcata</i> Wendl.	—	—	14 3
	<i>C. perfoliata</i> L.	—	—	14 - 9
Div. 9.	<i>Ramuliflora</i>			
	<i>C. Peglerae</i> Schönl.	7	28	14 8
Div. 10.	<i>Setulosa</i>			
	<i>C. sedifolia</i> N. E. Br.	—	—	14 10
	<i>C. Schmidtii</i> Regel.	—	—	14 2
Div. 13.	<i>Rosularis</i>			
	<i>C. rosularis</i> Haw.	—	—	14 11
	<i>Crassula</i> sp., near <i>rosularis</i>	—	—	14 - 13
	<i>*C. orbicularis</i> L.	7	—	—
Div. 14.	<i>Turrita</i>			
	<i>C. nodulosa</i> Schönl.	—	—	56 23
	<i>- C. hemisphaerica</i> Thunb.	7	27	14 ✓ 6
	<i>- C. barbata</i> Thunb. —	—	—	14 ✓ 4
Div. 16.	<i>Arta</i>			
	<i>C. deceptrix</i> Schönl.	—	—	14 - 14
Sect. V. Sphaeritis				
Sect. VI. Globulea				
	<i>C. cultrata</i> L.	—	—	14 5
Sect. VII. Pyramidella				
	<i>C. columnaris</i> Thunb.	—	—	ca. 14 —

The material of *C. columnaris* was lost before the definite count for the species was made, but an approximate number of $2n=14$ was ascertained. Two species of *Crassula* not mentioned by Berger (1930) were also investigated: *C. rotundifolia* Haw., $2n=14$ (Fig. 7), and *C. nana* Schönl. & Baker, $2n=34$ (Fig. 20). *C. coccinea* L., considered by Berger to be *Rochea coccinea* (L.) DC., has $2n=14$ (Fig. 1).

Fig. 14 is a drawing of late prophase. Figs. 1-26, exclusive of Fig. 14, are drawings of metaphases. Fig. 27 shows seven bivalents at 1st metaphase of a pollen mother cell division in *C. hemisphaerica*. Meiosis in this species is regular. Fig. 28 presents the metaphase chromosomes

($n=7$) of a pollen division in *C. Peglerae*. At this stage in this species the chromosomes are characteristically arranged in a single line; little deviation from this "rowed" alignment was observed in about a hundred pollen grains examined.

The range in $2n$ numbers is wide within this genus: 14–ca. 148. This latter number is the highest yet reported for the family. Since the plate from which the final count was made required a minimum amount of interpretation, 148 is probably correct. This number, however, falls into few regular series and is divisible by neither 7 nor 8: which numbers seem to be basic for two lines of evolution in the genus. In some of the species, those represented by Figs. 13 and 20 for example, the members of the chromosome complement are so morphologically different that the individual chromosomes can be distinguished without difficulty. Among the higher polyploids, no members of the complement can be recognized with certainty. In comparison with the diploid species ($2n=14$ or 16), the polyploid species *C. sarcocaulis* (Fig. 19) and *C. nana* (Fig. 20) show little or no reduction in the size of their chromosomes. Some of the diploid species (Figs. 17 and 18) have smaller chromosomes than these polyploid species.

DISCUSSION

That an attempt to arrive at the basic chromosome number of a taxonomic group from consideration of a small fraction of the species within that group might lead to erroneous conclusions was emphasized by Babcock (1934). The validity of that emphasis is appreciated. Though only about 10 per cent of the *Crassula* species have been studied, a peculiar cyto-taxonomic situation seems to justify at the present time certain conclusions with reference to basic numbers in the genus. Schönland, as referred to by Berger (1930), regards Tillaeoideae as the oldest section of *Crassula*. Fröderström (1929) retains the generic rank of Tillaea which largely constitutes Tillaeoideae and considers it to be the probable origin of the historically known Crassulaceae: "The annual species of this genus are scantily distributed over almost the whole globe, whereas the rest of the genera of the family are more or less limited to certain regions; the floral characteristics in this genus represent also the prototype of the real or apparent abortions in the flower, which occur in the rest of the genera of the family..." It is significant, in view of these attitudes toward Tillaeoideae, that this section appears to be founded upon a basic number of 8 while the remainder of the genus appears to fall, for the most part, into a 7 series. This situation is re-

vealed by an analysis of the list of the investigated species as given above.

Chromosome numbers for four species from three of the nine divisions of section Tillaeoideae have been determined. Each of these species has 16 somatic chromosomes; the presence of an 8 series is thus suggested.

Five species from four of the six divisions of section Stellatae have been investigated. Their chromosome numbers do not form a regular series. Numbers divisible by 8 ($2n=16$, *C. cordata*) and 7 ($2n=42$, *C. arborescens*) are present. Stellatae may be transitional between an 8 series in Tillaeoideae and a 7 series, as discussed below, in Campanulatae. Some of the species in Stellatae, however, fit neither series. *C. spatulata* could be regarded as a secondary polyploid of a 21-ploid condition of the 7 series, but since the other species, *C. sarmentosa*, in the same taxonomic division has ca. 60 chromosomes, such a hypothesis is at present unwarranted. *C. multicava* with about 112 chromosomes would fit into either of the two series mentioned; the high chromosome number and a similar chromosome size and morphology indicate the relationship of this species to *C. spatulata* and *C. sarmentosa*. Parenthetically, it is suggested that an increase in number of chromosomes may occur in roots growing out from the callus tissue that often results from propagation by cuttings. Rather frequent instances are on record of such a change in number of chromosomes in buds that arise from callus tissue.

In section Tuberosae, *C. nemorosa* is the only species that has been studied cytologically. Meiosis in this species is irregular; a definite n number was not determined. The section is not separated into divisions.

Numbers for fifteen species from nine of the seventeen divisions of section Campanulatae have been determined. Two other species, *C. rotundifolia* ($2n=14$) and *C. nana* ($2n=34$), should probably be included in this section. If these two species are included, the following series founded upon a basic number of 7 is present: 14-28-34-48-56. The frequency of distribution for these forms would be: 13 diploids, 1 tetraploid, 1 pentaploid (a derivative of 35), 1 heptaploid (a derivative of 49), and 1 octaploid. The species with 34 and 48 chromosomes could be included in an 8 series, but because of the morphology of their chromosomes and their intimate taxonomic relationship to the members of this 7 series, they are more logically placed in it.

No species from section *Sphaeritis* and only one each from section *Globulea* and section *Pyramidella* have been studied. A determination of $2n=14$ for each of these two species gives evidence of a possible 7 series in these two sections.

Representative species from *Crassula* have been investigated; some correlation with the taxonomy of the genus is exhibited by the chromosome numbers determined. The data, therefore, appear to warrant the following conclusions. If, as suggested by Fröderström (1929), *Tillaea* is restored to the rank of a genus, the primary basic number of *Crassula* is 7. If *Tillaea* is included within *Crassula*, the primary basic number of *Crassula* must be considered to be 8, and in the evolution of the genus a descending chromosome series of 8-7 has functioned with the secondary development of a predominant multiple series of 7-14-28, etc. The descending series is "usually intergeneric and seldom inter- and intra-specific"; the multiple series is usually "intra- and interspecific, rarely intergeneric" (Wanscher, 1934). Wanscher's observations favour the retention of *Tillaea* as a genus. It is so recognized in *Index Kewensis* where it has assigned to it fifty-five accepted species.

As established by Berger (1930), the subfamily Crassuloideae includes besides *Crassula* four restricted genera: *Rochea* with four species, *Dinacria* with three species, *Vauanthes* with one species, and *Pagella* with one species. *Rochea coccinea* has 14 somatic chromosomes. From this evidence, and since the genus consists of only four species, it may be assumed that *Rochea* has a basic number of 7. None of the other three genera have been investigated. The most ancient members of this subfamily belong to section Tillaeoideae of *Crassula* and have basically 8 chromosomes; the primary basic number of Crassuloideae must, therefore, be concluded to be 8, from which a more evolutionally effective number of 7 has originated. Consequently, in this subfamily a primary basic number of 8 has been lowered to 7. Within the closely related families, Saxifragaceae and Rosaceae, a basic number of 7 has been raised to 8 in the subfamilies Saxifragoideae and Rosoideae (Schoennagel, 1931).

The present data do not allow the formulation of definite conclusions concerning the origin of the family. A number of chromosomal series have contributed to its development; it may be polyphyletic in origin. *Crassula* possesses at least two series. Skovsted (1934), after determining the chromosome numbers of fifteen species from five genera of this family, states that "not less than five different chromosome numbers or series have been found". Investigations so far made on *Sedum* (about 10 per cent of the species have been studied) reveal an *n*-chromosome range from 6-64 with twenty-six intermediate numbers; more than one line of chromosomal evolution has been operative in the development of the genus (Baldwin, 1936). A detailed analysis of section Tillaeoideae will perhaps give an insight into the initial evolution of the Crassulaceae.

SUMMARY

Chromosome numbers for twenty-six species of *Crassula* are reported in this paper. Numbers for three other species have been reported by another investigator. The $2n$ -numbers exhibit a range of 14–ca. 148. This latter number is the highest known for the family.

Tillaeoideae is the oldest section of the genus. The species counted from this section have 16 somatic chromosomes. The other species are distributed taxonomically throughout the rest of the genus and belong predominantly to a 7-chromosome series: 14–28–35–42–48–56. It is assumed that if Tillaeoideae is restored to generic rank as *Tillaea*, the primary basic number for *Crassula* is 7 and, if Tillaeoideae is retained as a section, the primary basic number of *Crassula* is 8 although most of its species belong to a 7 series.

The subfamily Crassuloideae includes besides *Crassula* four small genera. Only one species from these four genera has been examined; *Rochea coccinea* has 14 somatic chromosomes. *Rochea* is assumed to have a basic number of 7. In the evolution of this subfamily a descending chromosome series of 8–7 has functioned, and a multiple series of 7–14–28, etc., has arisen secondarily and dominated the development of this taxonomic group.

No conclusions relative to the origin of the Crassulaceae can be definitely reached at the present time, but the chromosomal studies so far made indicate that in the development of this family several lines of chromosomal evolution have been operative.

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CROSSING-OVER AND ITS MECHANICAL RELATIONSHIPS IN *CHORTHIPPUS* AND *STAURODERUS*

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(With Plate XIII and Thirty-seven Text-figures)

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INTRODUCTION

CHROMOSOME behaviour has been more extensively studied in the Acridinae than in any other group of animals, except *Drosophila*. Janssens, Belar and Darlington and Dark have devoted special attention to *Chorthippus* and *Stenobothrus*. My object in the present study is to make use of this excellent material to test the conclusions that I have recently arrived at with regard to the mechanism of crossing-over (1935). I have carried out studies on the origin and behaviour of chiasmata in *Chorthippus* and elsewhere in order to find out what value the observation of a chiasma has at any stage of the prophase of meiosis in inferring the occurrence of crossing-over. These studies were quantitative, and they have made it possible to infer a one-to-one correspondence between crossing-over and chiasma formation and also to formulate a hypothesis of the mechanics of crossing-over. I can now carry the analysis a step further by qualitative study of chromosome arrangements, first in the nucleus before crossing-over, secondly in the diplotene nucleus after crossing-over, and thirdly at still later stages when crossing-over has taken place in inversions and in unequal bivalents.

The grasshoppers used were of the species *Chorthippus parallelus* and *Stauroderus bicolor*, the progeny of wild individuals caught near Guildford, Surrey, and in the grounds of this Institution, and described by Sansome

and La Cour (1935). I am indebted to my colleagues for the use of their material.

Smears were most suitable for the diplotene stages, sections 20μ thick for all others. Medium Flemming, Navashin and 2 BD fixatives (La Cour, 1931) gave similar and satisfactory results. Newton's gentian violet proved better than haematoxylin for all stages.

I. THE CHROMOSOME COMPLEMENT AT MITOSIS AND MEIOSIS

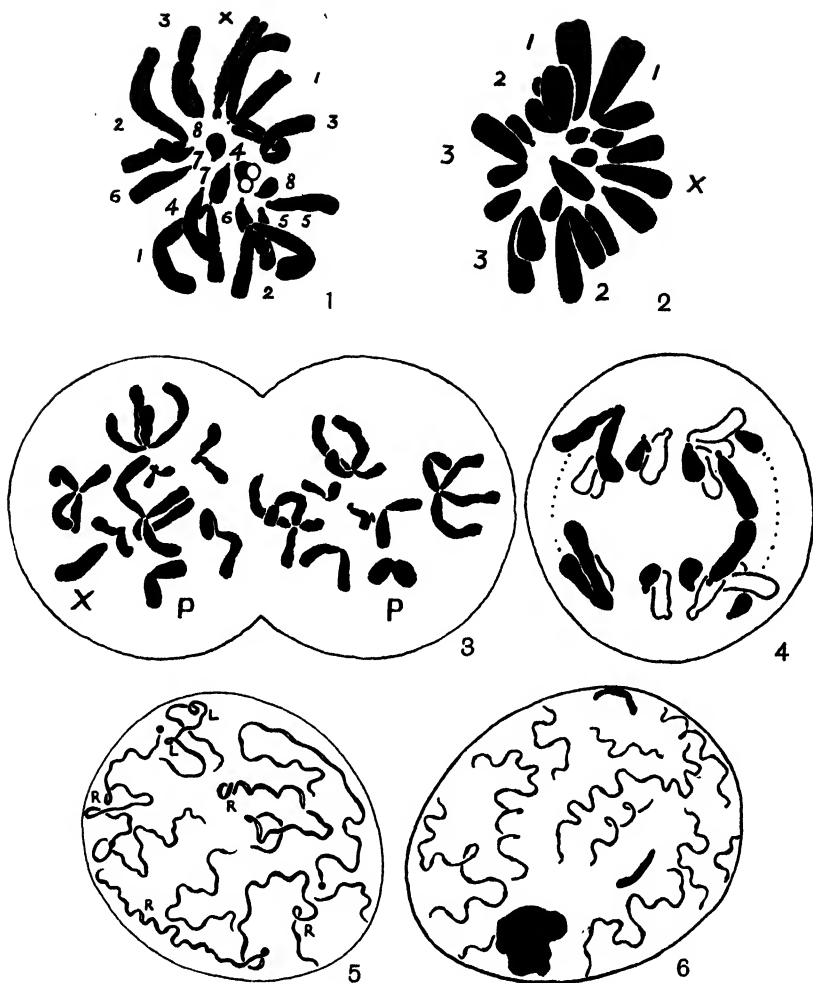
There are eight pairs of autosomes and one X-chromosome in the males of *Chorthippus parallelus* and *Stauroderus bicolor*. The chromosomes and the nuclei of the second species are smaller at all stages, and some individuals of this species also have a lower chiasma frequency at meiosis. The two are otherwise closely comparable.

The degree of contraction (spiralisation) of the chromosomes at metaphase differs in two ways. First, in different spermatogonial cells of the same testis with the same treatment (Text-figs. 1 and 2). In some they are even more contracted than in meiosis. This difference no doubt depends on developmental conditions. Secondly, in meiosis the degree of contraction was found to be reduced in a group of cells in *Stauroderus*. These cells had a slightly reduced chiasma frequency (Text-fig. 35). Such a difference is probably the result of a gene mutation, as has been shown in *Matthiola* (Lesley and Frost, 1927), and inferred in *Fritillaria* (Darlington, 1936*b*), *Tradescantia* (Darlington, unpublished), *Lilium* (Mather, unpublished) and *Tulipa* (Upcott, unpublished).

The prophases of mitosis and the leptotene stage in meiosis show characteristic relic spirals of regular direction which appear to have the same history as in plants (Text-figs. 5 and 6).

The chromosomes of the Acridinae to which *Chorthippus* and *Stauroderus* belong have always been described as of two types, with submedian and with terminal centromeres (or spindle attachments). The more easily studied structure of plant chromosomes has long suggested that apparently terminal centromeres would be found to be intercalary on closer study (cf. Lewitsky, 1931). I have found evidence of two kinds showing that this is true in *Chorthippus* and *Stauroderus*. First, small heads, or short arms, are visible at metaphase in spermatogonial divisions (Text-figs. 1 and 2) and even more clearly at the second metaphase of meiosis (Text-figs. 3 and 4). Secondly, as will be seen later, these short arms form chiasmata occasionally, giving characteristic configurations at first metaphase.

A small body, presumably the centromere, may sometimes be seen at



Text-figs. 1-6. *Chorthippus parallelus*. $\times 2400$.

Text-figs. 1 and 2. Spermatogonial mitoses with chromosome types numbered, showing different degrees of spiralisiation and differential behaviour of *X* with lower spiralisiation. The centromere is visible on some *L*-chromosomes in Text-fig. 1.

Text-fig. 3. Early second metaphase, showing differential condensation of *P*, and secondary constriction and trabants on *L*-chromosomes.

Text-fig. 4. Second anaphase, showing subterminal centric constrictions of *M*-chromosomes as in Text-figs. 1 and 2. *X* lagging.

Text-fig. 5. Early spermatogonial prophase with relic coiling.

Text-fig. 6. Leptotene with similar coiling; *X* condensed.

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metaphase of mitosis and second anaphase of meiosis at the centric or "attachment" constriction. Slight secondary constrictions may also sometimes be seen at second metaphase (Text-fig. 3). One of these is near the end of a long chromosome. It may be the result of the variable attachment of a small nucleolus which is seen during the prophase at or close to one or both ends of a chromosome of this type (the nucleolar chromosome, *N*).

The eight pairs and one unpaired chromosome may accordingly be classified for particular purposes in the following way in both species (cf. Darlington and Dark, 1932; and Darlington, 1932 *a*):

Position of centromere	No.	Length μ	Designation	Approx. chiasma frequency	Properties
Submedian	1	13	Long, L_1	3.8	—
"	2	12	Long, L_2	3.5	—
"	3	9	Long, L_3 or N	2.8	Nucleolar
Subterminal	4	5	Medium, M_4	1.7	—
"	5	4	Medium, M_5	1.5	—
"	6	4	Medium, M_6 or P	1.3	Very precocious (sometimes deficient)
"	7	3	Medium, M_7	1.1	Precocious
"	8	1.6	Short, S	1.0	Precocious
"	9	6	(Med.) X	—	Sex-chromosome, unpaired in male

II. POLARISATION AND DIFFERENTIAL CONDENSATION

In the heterozygous sex of *Chorthippus* and *Stauroderus* the *X*-chromosome is often under-condensed at metaphase in the spermatogonial divisions. It resembles in this respect the sex chromosomes of the rat in meiosis (Koller and Darlington, 1934). It is on the other hand over-condensed in the meiotic prophase from leptotene to diakinesis. Certain other chromosomes share this property in varying degrees. One of the medium chromosomes (*P*) is regularly and completely over-condensed or "precocious" at late pachytene and early diplotene. M_7 and S and to a less extent the *L*-chromosomes are also over-condensed locally, near their ends. This is clearest at early diplotene (Text-fig. 22). The *P*-chromosome can be recognized by its over-condensation even at second metaphase, although the *X* cannot (Text-fig. 3). *X* is the inverse of the ordinary chromosomes in staining capacity at different stages of meiosis; *P* is not.

The properties of chiasma formation of the *P*-chromosome differ from those of the other chromosomes in no way except in timing. Chiasmata appear earlier, on account of the earlier separation of the chromosomes, but with corresponding frequency and distribution. If the condensation

of the *P*-chromosome meant a difference in structure this could not be so; the materials associated with it to give the appearance of condensation must therefore be irrelevant to its structure; they must be merely adsorbed on its surface.

Some light is thrown on this behaviour by the movements of the condensed chromosomes. *P* is precocious in diplotene separation (Text-figs. 17, 21 *a*). *X* is bent back on itself at pachytene and gradually unbends during diplotene (Text-fig. 20). As it does so, it loses its excessive condensation, and at the same time the small nucleoli attached to the various other chromosomes disappear. It is as though the *X*-chromosome had been submerged in nucleolar material which, when it dissolves, releases the chromosome, and allows it to straighten itself out like the rest. In order to understand this behaviour we have to consider the movements of all the chromosomes in the course of polarisation, for during this process the condensed chromosomes arrange themselves differently from the rest.

Polarisation during the prophase of meiosis consists in the orientation of the chromosomes in relation to a pole which is probably determined by the centrosome. It must be distinguished from the telophase orientation of the centromeres towards the pole in mitosis. It must also be distinguished from the fusion of all the centromeres to form a central magma in the salivary gland cells in *Diptera*. Polarisation has been described in many plants and animals (cf. Wenrich, 1916; Gelei, 1921) and in especially great detail in *Chorthippus* and *Mecostethus* by Janssens (1924). By comparing the different arrangements of all the chromosomes in different nuclei it is now possible to analyse the dynamics of their very variable behaviour. The behaviour of the two species seems to be closely comparable in spite of the difference of size between their chromosomes and their nuclei.

The simplest arrangement is that where the chromosomes are arranged with all their ends, except perhaps one or two, towards the pole (Text-fig. 7). The condensed *X*-chromosome is usually lying close to the pole but not so close as the ends of the others. (It sometimes lies on the opposite side of the nucleus.) This arrangement is modified in most nuclei by a variable number of the ends of the other chromosomes being attached to the *X*. The commonest aberrant in this respect is *P* (the "dyade compagnon" of Janssens) and the second commonest is *S* (Text-figs. 12-16). These are the two most condensed chromosomes. Their association is not specific to their parts or to the parts of the *X*, for occasionally it may be seen to be intercalary with respect to either of the

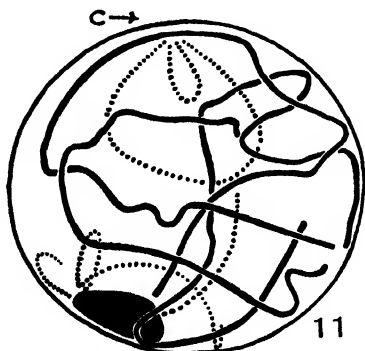
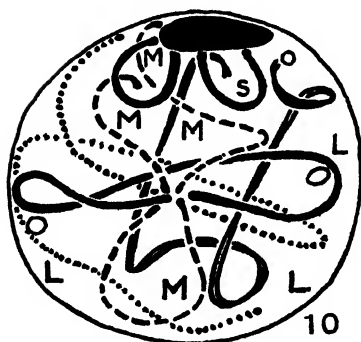
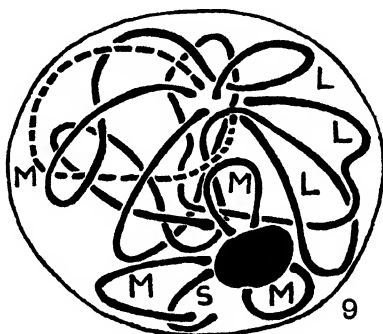
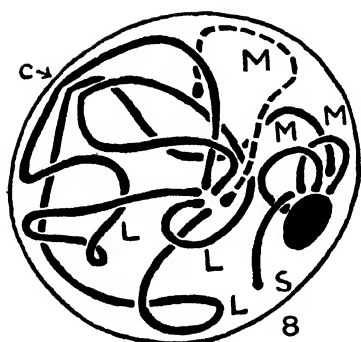
partners, either at pachytene (Text-figs. 11 and 16) or at diplotene (Text-fig. 20 *g, j, m*).

Of the other chromosomes, both *L* and *M* types associate less frequently with *X* and only at their ends, which may themselves be over-condensed. Perhaps certain chromosomes are never over-condensed even at the ends and are entirely unattracted by the *X*, but since every chromosome pair cannot be recognized, and no two nuclei are alike, we can only suppose that this may be so. It may happen to a chromosome of any type that one of its ends is associated with the *X*, the other with the pole. There is also evidence in two nuclei of a possible third centre of attraction—the centromeres of the *L*-chromosomes. In one nucleus the three are lying close together (Text-fig. 8 *c*) and in the other, one of them seems to have attracted the ends of two *M*-chromosomes (Text-fig. 11 *c*). The centromeres, however, seem to have no influence on the ordinary process of polarisation. The two ends of an *M*-chromosome, one of which is close to the centromere, the other remote from it, do not seem to be distinguishable in their behaviour.

As the nucleus passes from pachytene to diplotene the polarisation *relaxes*. But it is a mistake to suppose, as previous workers have done, that the differences in arrangement of the chromosomes at these stages (as shown in Text-fig. 20) can be put in a single seriation and are merely due to differences of time. On the contrary, those early diplotene figures in which the three precocious chromosomes are attached to the *X* and to one another are the ones where they have been so attached at pachytene, and those in which they are free are the ones that have had a single pole at pachytene with a free *X*, as shown in Text-fig. 7.

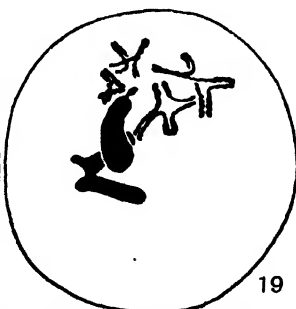
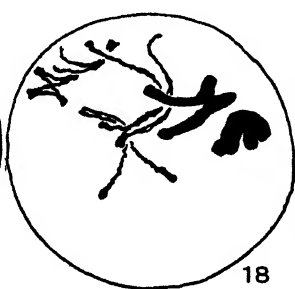
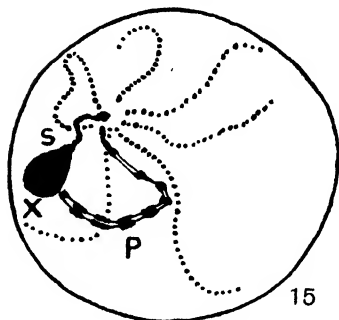
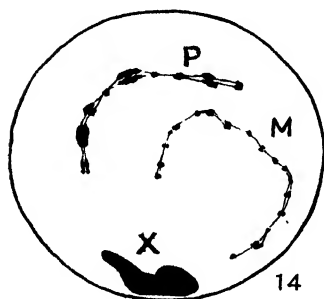
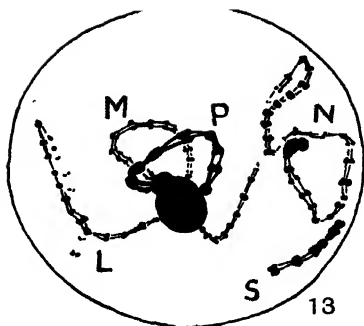
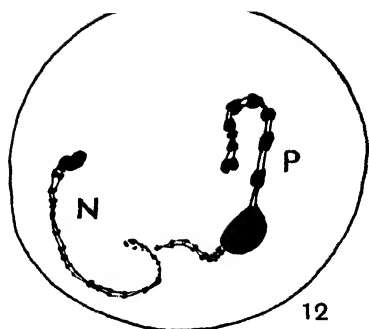
The association of chromosomes with the *X* has many properties in common with nucleolar fusion such as is found particularly at the prophase of meiosis in plants. It occurs between parts of chromosomes which themselves appear to be embedded in nucleolar substance or to bear nucleoli at the ends which fuse, and it persists as long as the nucleolar appearance persists. But what is most significant is that chromosomes which bear small nucleoli terminally or even laterally may become associated with one another owing to the fusion of their nucleoli (Text-figs. 16 *S*, 28 *c*, Pl. XIII). This is relatively rare, as would be expected if the force of attraction is a function of the size of the bodies concerned. Two small nucleoli fuse less frequently than one small nucleolus fuses with the large *X*.

The variable orientation of ends towards one or the other of the two main centres of attraction suggests that it results from a chance variation



Text-fig. 7. Diagram of a complete mid-pachytene nucleus in *Chorthippus* with all chromosomes simply polarised at both ends, except one. *X* and part of *P* condensed. Three *L*-chromosomes shown dotted, broken, and wavy. $\times 4800$.

Text-figs. 8-11. Diagrams of four complete pachytene nuclei in *Stauroderus*, showing variable attraction of the autosomes to *X* and to one or two poles. $\times 3000$.

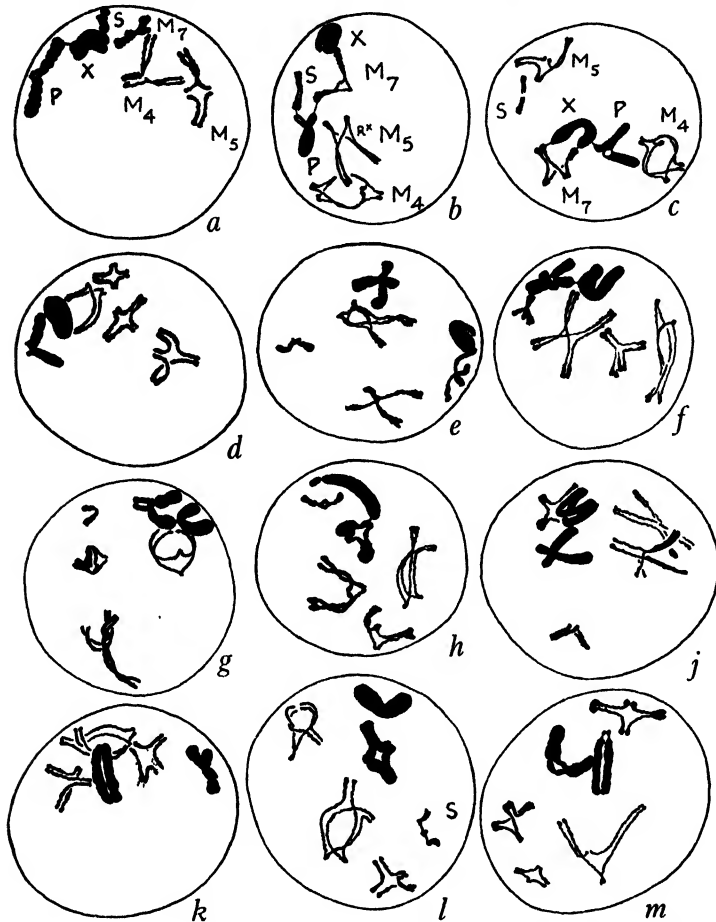


Text-figs. 12-15. Stages in polarisation in *Chorthippus*, particularly of *P* and *S* chromosomes. *N*, an *L*-chromosome which usually bears a nucleolus at one or both ends. $\times 2400$.

Text-fig. 16. The varying appearance of *P* when attached to *X* at pachytene in *Chorthippus*. *S*, the short chromosome forming a ring owing to fusion of its condensed ends. $\times 2400$.

Text-figs. 17-19. *X*, *P*, *S* and the three *M* pairs at the beginning of diplotene in *Chorthippus*, showing precocious opening of the *P* pair (cf. Text-fig. 21). $\times 2400$.

in the spacing of the chromosomes, those chromosome ends which happen to lie near the *X* fusing with it while others move towards the pole. The fusion of the chromosomes with the *X* will impede its movements and



Text-fig. 20. The complement apart from the *L*-chromosomes in twelve early diplotene nuclei. These chromosomes are always grouped as a result of polarisation, and where they seem spread are on one side of the nucleus. *P*, *S* and *M₇* frequently remain attached to the *X* and to one another (as chromosomes are attached to nucleoli at this stage in plants). Note the gradual opening of the *X*. $\times 1600$.

thus account for the variation in its own position, and for its failure in many nuclei to reach the neighbourhood of the pole towards which it also is attracted.

The relationship that I am here suggesting between precocious condensation and the deposition of nucleolar material brings us back to the

old name of "chromatin nucleolus" which was abandoned when the genetic function of the X-chromosomes was recognized. This description has long seemed extravagant, but it is made more plausible by some apparently unrelated observations on *Zea Mays*. McClintock (1934) showed that the deposition of nucleoli which is ordinarily controlled by local centres or "organisers" may under abnormal physiological conditions occur evenly over the chromosomes, which become embedded in nucleolar substance. These abnormal conditions were determined by genetic unbalance in McClintock's case. May we not equally suppose that at special stages in the cell history of the grasshopper of a special genetic constitution (viz. the male) nucleolar material is deposited on certain chromosomes in a similar way?

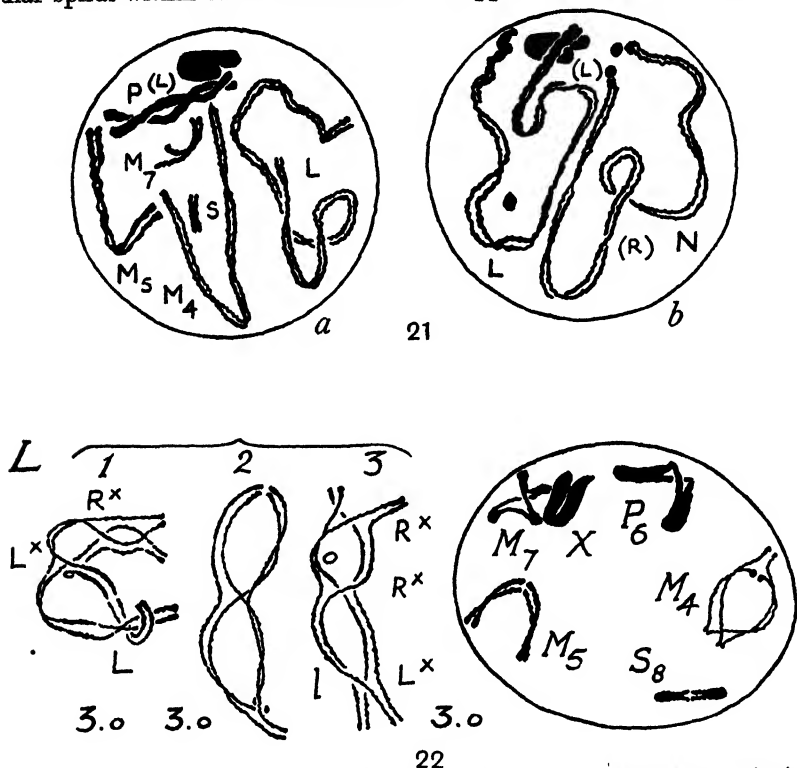
Conclusion. The essential properties of polarisation in *Chorthippus* and *Stauroderus* therefore seem to be due to the action of two forces of attraction, (1) between all chromosome ends and the pole, (2) between all condensed or nucleolar parts of chromosomes, manifesting itself chiefly in the fusion of these parts with the X. The differences that result are due simply to differences in the relative positions of the chromosomes in the nucleus before polarisation.

When these forces are considered in relation to those governing the external movements of the chromosomes at other times they seem to be exceptional in being non-specific forces of attraction; all other attractions are specific (Darlington, 1936 b). There is no evidence even that the attraction of terminal affinity, shown by the fact that chiasmata do not slip off the ends during terminalisation, is other than specific. It is peculiar to all ends of chromosomes, but it is only shown between homologous ends, and seems to depend merely on their position. There are, however, two ways of considering the polarisation attraction as specific. One is by supposing, as Haldane has suggested (1936) on the basis of an analysis of structural changes, the ends have certain specific properties of which one is the property of not joining other ends as broken middle parts join one another. The other is by supposing that this attraction is, like that between condensed chromosomes, conditioned by the presence of nucleolar substance. Further evidence is necessary to distinguish between these two possibilities.

III. COILING AND CHIASMA FORMATION

I have recently attempted to describe the conditions that may be supposed to determine crossing-over. According to my hypothesis a change in the degree of coiling occurs in the molecular spiral of the paired

chromosomes during the pachytene stage, and this change sets up a relational coiling of the chromosomes, which may be seen (Text-fig. 21). The change is associated with a shortening of the chromosomes in the Orthoptera, a lengthening in the Mammalia and Liliaceae. The torsion of the relational coiling must be in opposition to the torsion of the molecular spiral within each chromosome. I suppose, but have not proved,



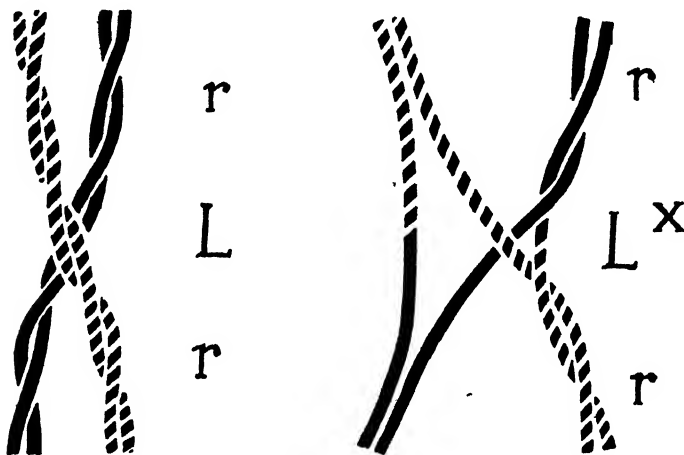
Text-fig. 21 *a, b*. Complete nucleus at the end of pachytene in *Chorthippus*, polarisation lapsing, relational coiling developed (*L* and *R*), *N*, nucleolar *L* chromosome. $\times 2400$.

Text-fig. 22. Complete early diplotene nucleus in *Chorthippus*. Numbers of total and terminal chiasmata and their direction (*R*^x and *L*^x) given for *L*-chromosomes. $\times 2400$.

that the cleavage surface of the chromosomes is such that it would be straight in a chromosome subjected to no external stresses at the end of pachytene. If this is so, the chromatids produced by division will be coiled round one another in the opposite direction to that in which the chromosomes are coiled round one another. Equilibrium will be reached between the two systems of coiling when they have a certain quantitative relationship (not necessarily equality) depending *inter alia* on the fre-

quency of coiling. Crossing-over is then due to breakage at the moment of division when the cohesion of the daughter chromatids is unequal to the strain imposed by the coiling of the parent chromosomes. Uncoiling of the broken chromatids releases the strain before they reunite in a new combination to give the cross-over chromatids.

Arising out of this general statement are several questions of detail which can be answered by studying material suitable for these special purposes at the stages immediately before and after crossing-over has taken place. Thus:



Text-fig. 23. Diagram showing the origin of a left-hand chiasma (L^x) from crossing-over following opposite relational coiling between chromatids (r) and between chromosomes (L).

(1) Are the chromosome and chromatid coilings opposite? If, for example, the division of the chromosomes is such that the potential chromatids would be parallel in a free chromosome at leptotene (and not at the end of pachytene), then the two kinds of relational coiling will be in the same direction.

(2) What amount and what proportion of coiling are used up in crossing-over?

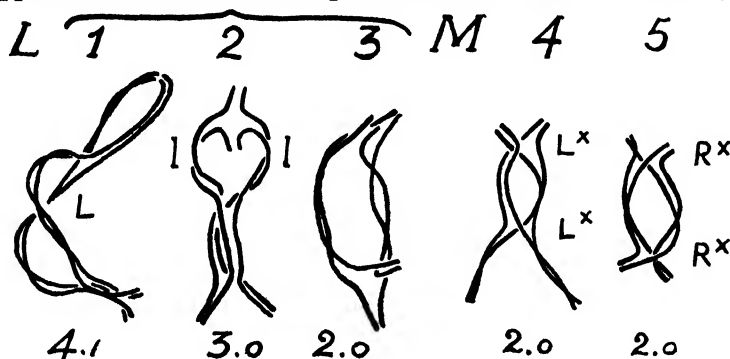
(3) Can the direction of coiling which has determined crossing-over be recognized by the spatial characteristics of the chiasma it produces?

(4) Can the cross-over chromatids be distinguished from the non-cross-overs in the newly formed chiasma?

(5) How do changing conditions during and after crossing-over affect the equilibrium position of chromosome and chromatid coiling? In

other words, what happens to the two kinds of coiling during the diplotene stage?

Some of these questions can be answered by a study of *Chorthippu*. The last is the easiest and it is necessary to consider them in reverse order. Relational coiling of chromosomes and of chromatids can always be seen in all the *L*-chromosomes at early diplotene and in some of the *M*-chromosomes. This coiling is always consistent in one arm of a chromosome. A loop including the centromere, however, shows opposite systems of coiling in one case (Text-fig. 25*l*), but later where they have been present the lesser has been cancelled by the greater. In opposite arms coiling was in opposite directions in all except 2 of the 14 bivalents recorded (Text-



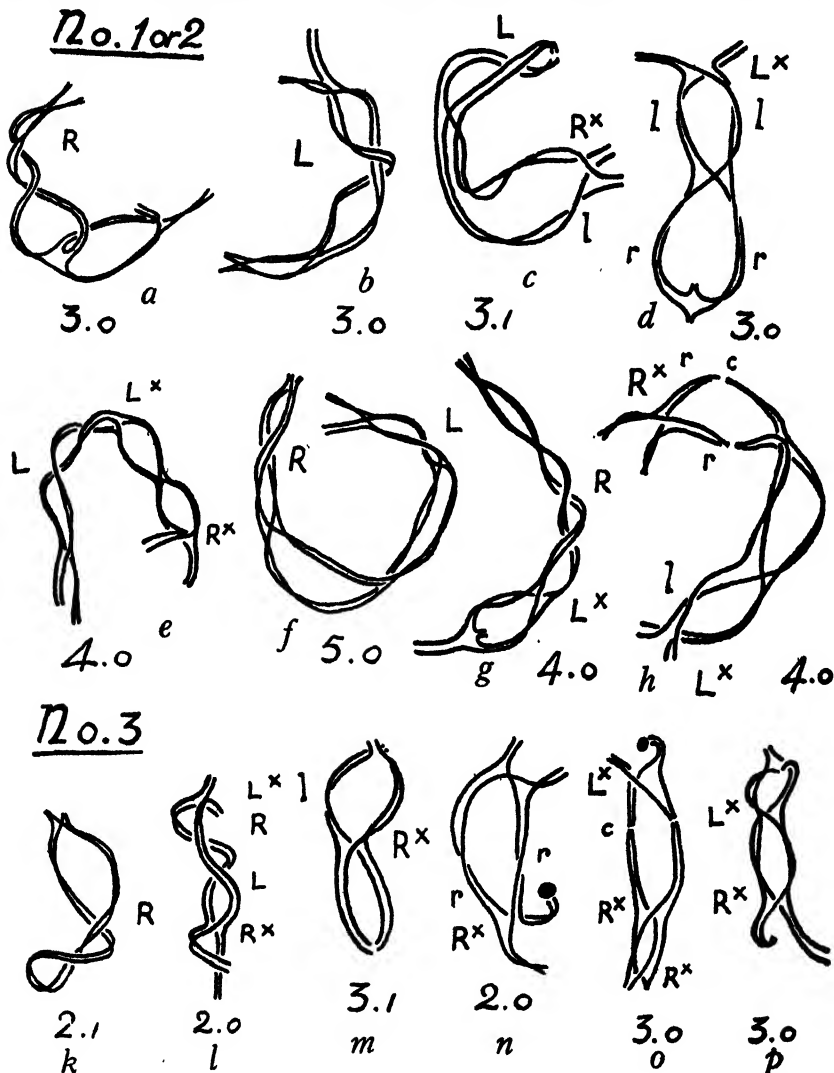
Text-fig. 24. The three *L*-chromosomes from one nucleus, *M*₄ and *M*₅ from other nuclei, showing the numbers and directions of chiasmata. $\times 2400$.

Note. Two difficulties arise in illustrating this coiling. First, a two-dimensional drawing will give the false appearance of this coiling in a symmetrical figure whenever it has been seen diagonally. Secondly, in genuine cases the general strain of relational coiling of chromosomes forces the two coiled chromatids as close together as possible, so that the direction of coiling cannot always be shown.

figs. 24–26). These exceptions are perhaps due to a transference of coiling from a coiled arm to an uncoiled one as in *Fritillaria* (Darlington, 1935 *d*).

The chromosome coiling distal to chiasmata is lost during diplotene by the uncoiling of ends, and that between chiasmata by further coiling of chromatids; the coiling of chromatids also is lost in the free ends distal to chiasmata but not between chiasmata. Chromatids thus come to lie parallel in all free ends, and this proves that the persistent overlaps between chiasmata are indeed regular coils and not fortuitous twists. Chromatid coiling is absent only in short loops where two cross-overs have taken place close together (Text-fig. 24) and sometimes in loops including the centromere, where the opposite directions of coiling on either side of the centromere referred to earlier have presumably cancelled one another out, as happens with chromosome coiling.

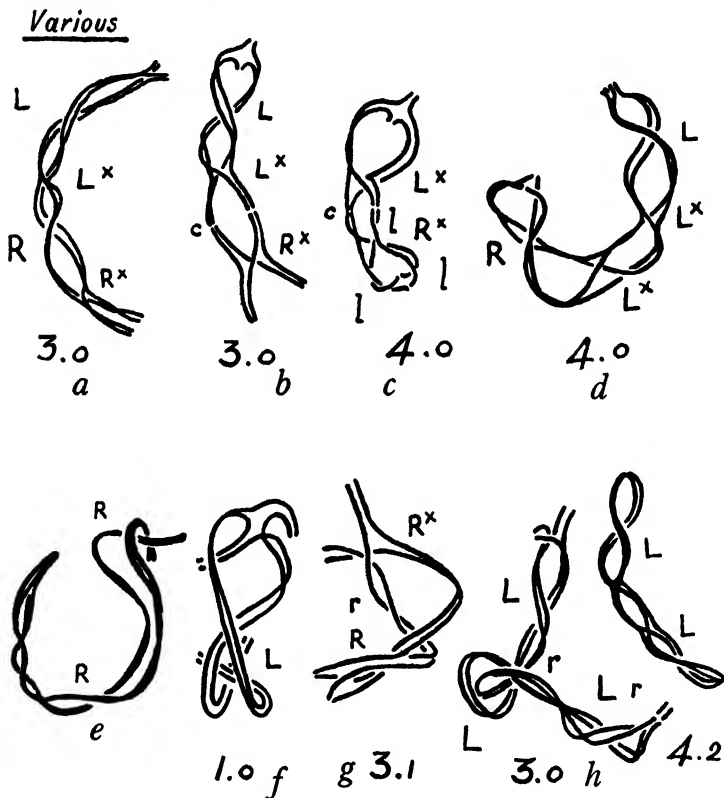
How can we now recognize the two cross-over chromatids at a chiasma? Successive loops usually lie in the same plane directly on either side of an



Text-fig. 25. Selected bivalents of No. 1 and No. 3 types showing diagrammatically the direction of coiling and chiasmata where ascertainable. Numbers of total and terminal chiasmata under each bivalent. *c*, the centric constriction. $\times 2400$.

early diplotene chiasma. There are then two *outer* and two *inner* chromatids at the chiasma. One of the inner pairs passes over the other; they are therefore coiled and their spiral relationship can be determined.

Chiasmata can then be said to have either a right- or a left-handed direction of coiling (R^x and L^x in the figures). On the hypothesis I have stated the outer chromatids are the cross-overs, while the inner chromatids represent by their coiling the surviving relational coiling of the chromosomes (Text-fig. 22). A wool model under the assumed conditions



Text-fig. 26. Unidentified *L* bivalents as in Text-fig. 25. *e*, a very early diplotene bivalent without chiasmata or relational coiling over half its length. $\times 2400$.

of stress gives the same result. The direction of chiasma and chromosome coiling should be the same. The direction of the chiasma was consistent in the five clear cases illustrated with the chromosome coiling. In one doubtful case (Text-fig. 25) it was inconsistent. The distinction of the direction of a chiasma lapses at a later stage, for then all the relational coiling of chromosomes disappears; the chiasmata become symmetrical, and merely separate successive loops at right angles to one another.

The *M*-chromosomes being shorter lose their relational coiling more quickly. Moreover, they usually form only one chiasma and they are

then in a position to lose all their relational coiling of chromosomes and chromatids by uncoiling at the four ends to give the characteristic symmetrical cross, preserving no trace of torsion (Text-fig. 20). Even in these, however, at the earliest diplotene the two open pairs of arms lie in the same plane, and two of the chromatids then have a coiling direction at the chiasma although they generally lie too close together to be visually separated. The *M* bivalents having subterminal centromeres consist effectively from the point of view of diplotene coiling of one arm. In two *M* bivalents with two chiasmata I was able to classify both the chiasmata, and these had the same direction, as would be expected from a consistent direction of relational coiling in one arm at pachytene.

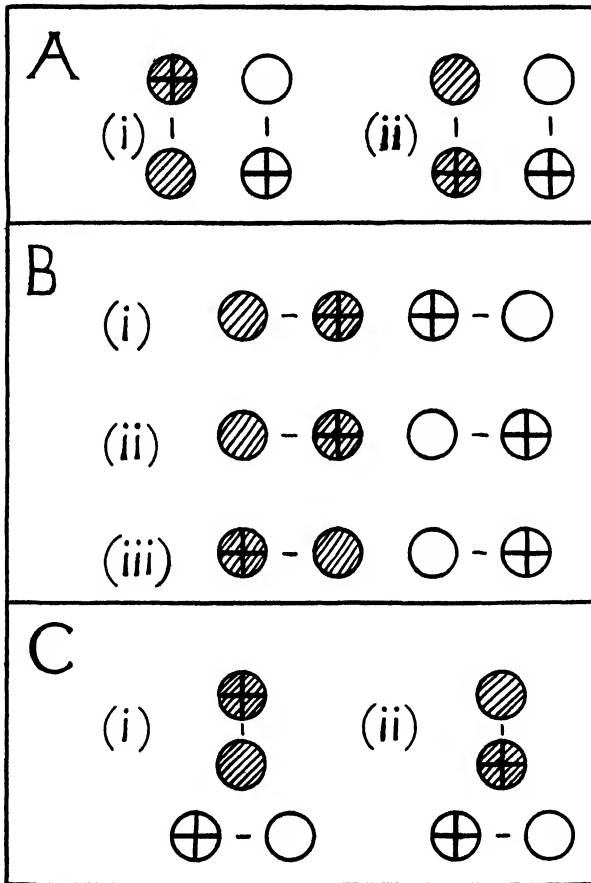
The direction of chromatid coiling is less fully ascertainable, especially when it has a high frequency, than that of chromosomes and chiasmata (cf. Note, Text-fig. 24). The results are consistent as regards the direction in the corresponding arms of five *L* pairs recorded. But they fail to be consistent in relation to the chiasma and chromosome coiling directions; they agree with these in eight cases, disagree in five. This inconsistency does not seem to depend on errors of observation but it might be due to special circumstances such as redistribution of strain in chromosome coiling. Further observations are therefore necessary.

In *L*-chromosomes with an average of three chiasmata the amount of relational coiling which survives at early diplotene between chiasmata varies between half a revolution and two complete revolutions. The chromatid coiling varies similarly. Neither is found between chiasmata that are very close together. Such considerations should enable us to estimate the proportion of coiling used up in crossing-over. This problem is not at present soluble for the following reasons.

The amount of relational coiling of chromatids left in the four arms of a chiasma is sometimes unequal. The result is an asymmetry of the chiasma (Text-figs. 21, 23). This type of observation shows that some of the four pairs of chromatids have lost more of their relational coiling at the time of crossing-over than others, assuming as we must, that the chromatids of the two chromosomes have been equally coiled at pachytene. Such a result is to be expected with the mechanism I have suggested.

Thus the two pairs of coiled chromatids may be spatially arranged in an infinite number of ways in relation to one another at the point of crossing-over. Three of the most distinctive types may be described as square, linear and *T*-type. In the first and last, crossing-over may be supposed to be possible in two ways, in the second in three ways (Text-

fig. 27). It is of course unlikely that these *a priori* possibilities are equally frequent. The observed frequencies of particular relationships of successive cross-overs must depend on their relative frequencies with regard to which we know nothing. Obligatory reciprocal crossing-over in



Text-fig. 27. Diagram showing the lateral relations of four chromatids at the moment of crossing-over in a system with opposite coiling of chromosomes and chromatids. A, square type; B, linear type; C, T-type. Chromatids of the partners open or hatched. Cross-over chromatids crossed. B (i) gives maximum uncoiling; C (i) gives minimum uncoiling; B (ii) and C (ii) give unequal coiling.

Drosophila probably depends on special conditions in this respect. But it is clear that in two, B (ii) and C (ii), of the seven classified spatial arrangements crossing-over will reduce the coiling of the two pairs of chromatids unequally and give rise to the observed asymmetry at diplotene.

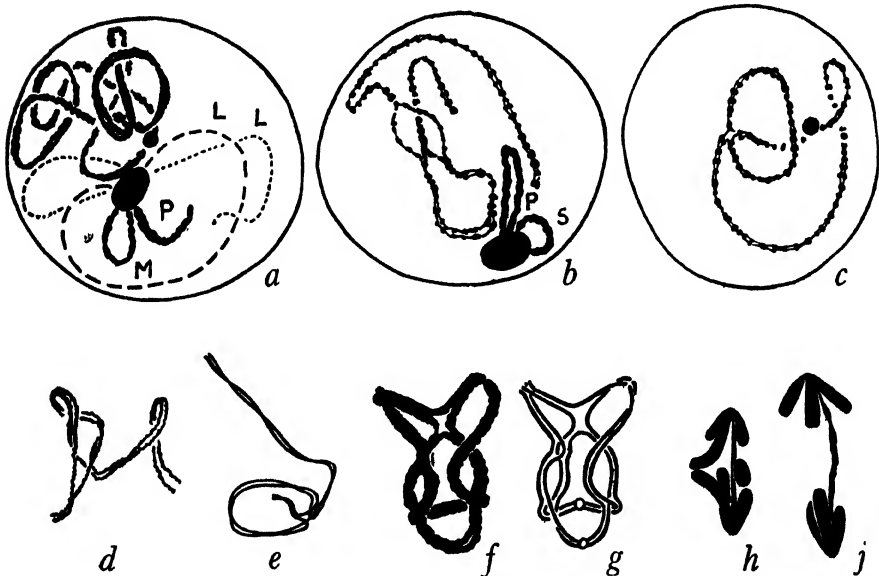
These considerations also show that the amount of uncoiling produced by a cross-over will depend on its spatial conditions. This variation is perhaps responsible in part for variation seen in the frequency and distribution of crossing-over. Experiments with wool confirm this expectation, in showing that crossing-over reduces the amount of coiling, measured in complete chromatid revolutions, c , by amounts varying from 3.5 to 7 c . It is not therefore possible to estimate from the present data in *Chorthippus* the proportion of coiling used up in crossing-over.

The consequences of chromatid coiling in organisms with complete terminalisation are worth considering. Just as coiling is lost in free distal segments, it must also be lost in the loops which are replaced by proximal ones in terminalisation as I have described it in other species (1932 *b*, Text-fig. 87). We know that terminalisation is a necessary adaptation for disjunctional arrangement in multiple rings and promotes regular and simultaneous disjunction in all cases. We now see that it is also indispensable for easy anaphase separation in an organism with a considerable amount of residual coiling of chromosomes and of chromatids at diplotene proximal to the most distal chiasmata. All the coiling of both kinds except that within the proximal loop will be lost when terminalisation is complete. This shows us an adaptive reason why coiling is so often in opposite directions on opposite sides of the centromere; it leads to cancellation of coiling in this important proximal loop. With terminalisation therefore the untwisting of chromatids will take place before metaphase instead of at anaphase. A single twist at anaphase leads to great tension in meiosis (Belar, 1929) and even in mitosis (Darlington, 1936 *b*). Several twists might therefore lead to rupture or a genuine non-disjunction such as has never been observed.

Although a single coil in a loop should be readily uncoiled in terminalisation, the possibility presents itself that the strain of undoing several coils might lead to the breakage of one of the terminal associations before metaphase. This would account for the "imperfect" terminal chiasmata that have been seen in *Tradescantia* and *Primula sinensis* (cf. Darlington, 1932 *b*).

Conclusion. The existence of three kinds of coiling—of chromosomes, chromatids and chiasmata—immediately after crossing-over has taken place, and the changes that these kinds of coiling undergo, show that the chromosomes have been in a state of coiling stress. On any mechanical hypothesis compatible with the spatial relationships observed, crossing-over must reduce coiling stress. We must therefore suppose that it removes only a part of the pre-existing stress which determines its occur-

rence. The spatial arrangements of the four chromatids before crossing-over cannot be inferred with certainty from the present observations, but the pair of chromatids at the chiasma, that have crossed over can be distinguished: they are the outer pair, since these are less coiled than the inner pair which still show the direction of relational coiling by their relative position. Their residual coiling is lost when successive loops come to lie at right angles to one another.



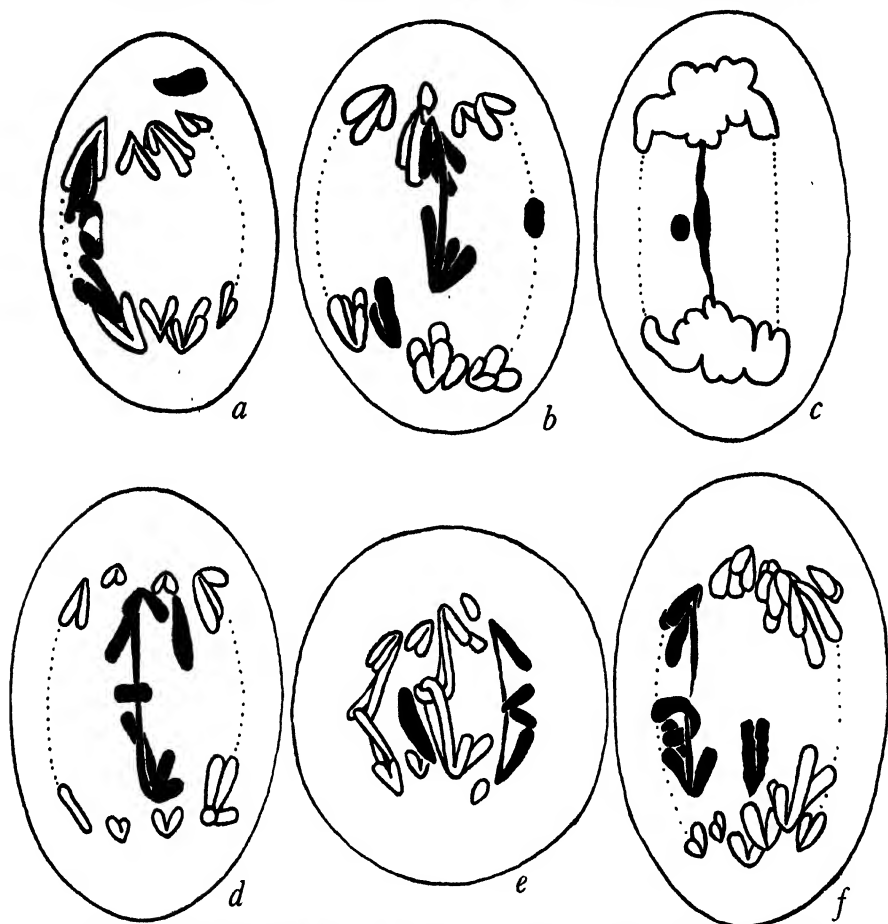
Text-fig. 28. The behaviour of relatively inverted segments at meiosis. *a-c*, pachytene pairing in *Chorthippus* 4. *a* and *c* show the fusion of the two nucleolus-bearing ends of the *N* bivalent which carries the inversion. *d*, the same in *Chorthippus* 6. *e*, in *Stauroderus* 6, which gives no dicentric and acentric chromatids owing to the inversion including the centromere. *f, g*, chiasma formed between two relatively inverted and translocated segments at diplotene in *Chorthippus* 8. *h, j*, bridge and fragment formed by the dicentric and acentric chromatids resulting from crossing-over between two relatively inverted segments in a dyscentric hybrid (the same individual). $\times 1600$, except *f* and *g*, $\times 2400$ (cf. Pl. XIII, fig. 10).

IV. CROSSING-OVER IN STRUCTURAL HYBRIDS

(1) *Dyscentric hybrids*

The pairing of chromosomes containing relatively inverted segments at the pachytene stage of meiosis has been described by McClintock in irradiated *Zea Mays* (1933). Its consequences at later stages have been analysed and described in terms of the accepted theory of crossing-over by Richardson in *Lilium* hybrids (1936). Evidence of their occurrence in a

natural population has also been found in species of various plants. *Trillium*, *Tulipa*, *Tradescantia*, *Fritillaria* and *Agave* (cf. Smith, 1935; Darlington, 1936 a), and they are known to distinguish natural races in *Drosophila* (Sturtevant, 1926; Sturtevant and Dobzhansky, 1931; Painter,

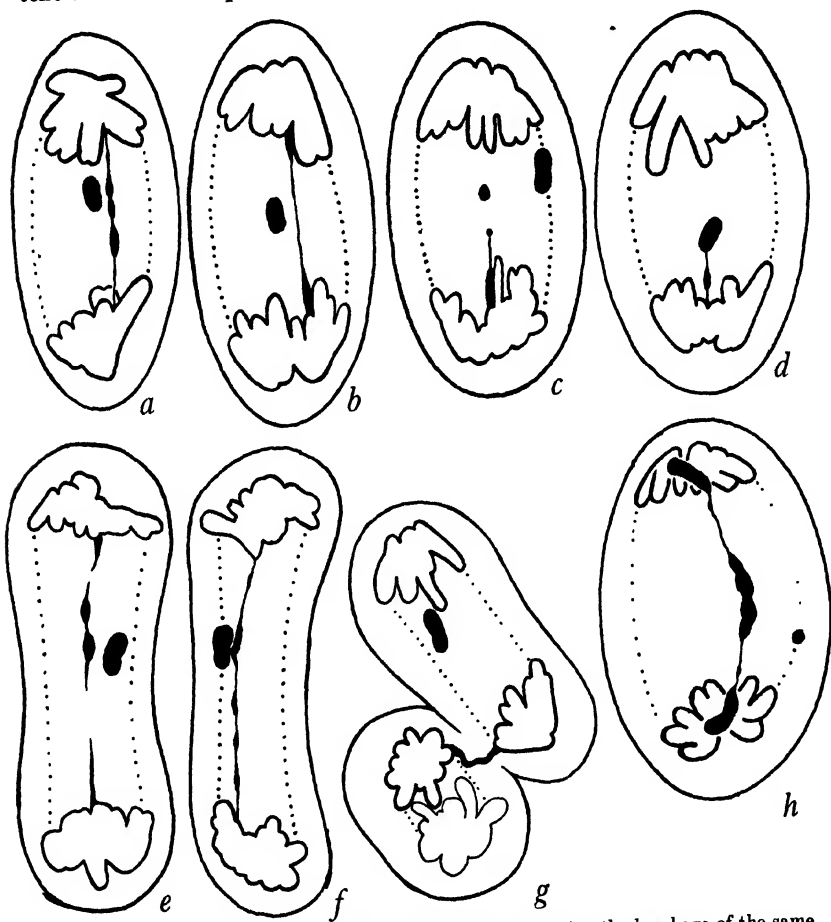


Text-fig. 29. First anaphases in *Chorthippus*, showing chromosome with bridge and fragment and X-chromosome black. a, No. 3 (smear preparation), bridge not visible, very short and therefore probably broken. b, No. 5, characteristic free fragment on edge of spindle. c, No. 4. d, e, f, strangled fragments. d and f, No. 5. e, No. 6. $\times 2400$.

1934; Koller, 1935). It is therefore worth while finding out how frequent they are in other animals.

Five individuals of *Chorthippus parallelus* and four of *Stauroderus bicolor* were examined. All showed the presence of inversions except one individual of the second species. The evidence of the inversions is

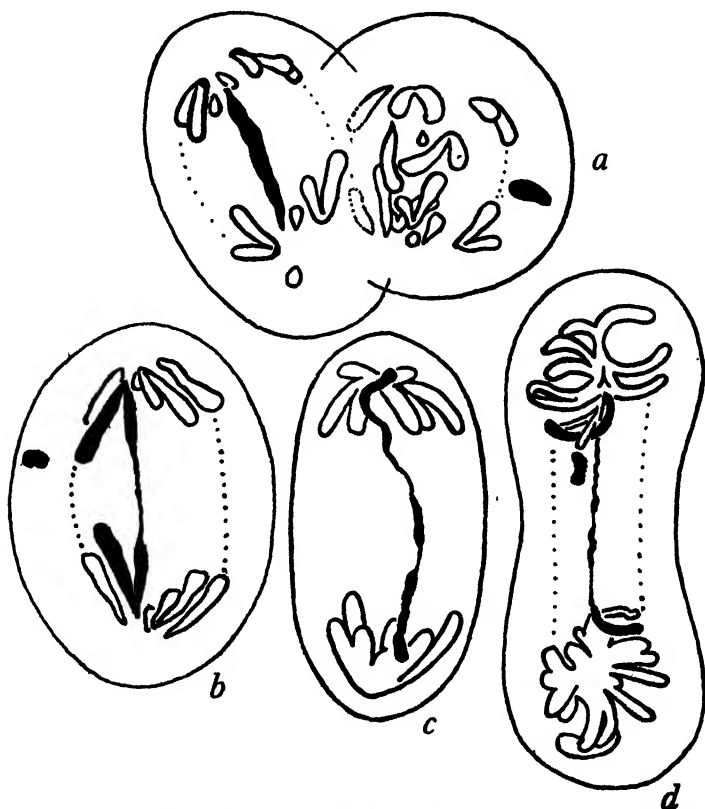
derived from three stages according to three consequences of the homologous chromosomes having differently arranged parts. First, at pachytene we find the simple side-by-side pairing interrupted by the occurrence



Text-fig. 30. *a-f*. Late first anaphase in *Chorthippus 8*, showing the breakage of the same bridge in various places and the carrying of a strangled fragment towards one pole (*d*). *g*, the persistence of a first division bridge and fragment to the second division. *h*, first division bridge in *Stauroderus 2* (*M*-chromosome).

of reversed loops (Text-fig. 28 *e*). These loops are intercalary and not terminal. Secondly, at diplotene we find chiasmata resulting from crossing-over within the reversed loops. These are recognisable only when the crossing-over has taken place well away from the middle of the inversion so as to give asymmetry, or when translocation is also involved. One

individual of *Chorthippus* had a chiasma in one cell at diplotene such as would arise from crossing-over between segments relatively translocated within the chromosome and also inverted (Text-fig. 28 *f* and *g*, Pl. XIII, figs. 7 and 8). No similar configuration was found elsewhere in the same individual, this might be taken to argue that the inverted segment was



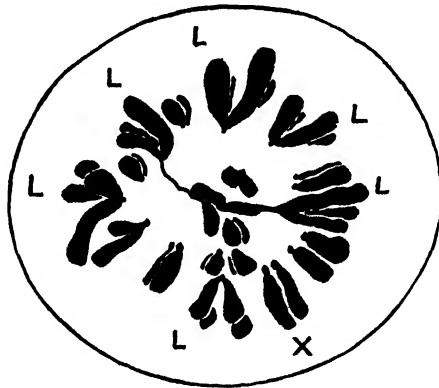
Text-fig. 31. Second division anaphase in *Chorthippus*. *a*, second division bridge from a first division loop in an *M*-chromosome, with the fragment off the spindle (No. 4). *b* and *c*, a second division bridge (Nos. 5 and 8, *L*-chromosome). *d*, later second anaphase (No. 8). $\times 2400$.

exceptionally short, but probably it indicates rather that the relatively translocated segments do not always pair at pachytene and therefore do not always get a chance to cross-over. It is worth noting that crossing-over in this way will give ring chromosomes of the kind found in *Crepis*, *Zea*, *Tulipa* and *Drosophila*.

Thirdly, at anaphase we see a consequence of crossing-over in inversions in the behaviour of the cross-over chromatids. One of these joins

two centromeres; it is *dicentric*. The other has no centromere; it is *acentric*. The dicentric chromatid forms a bridge between the two poles at anaphase, the acentric chromatid lies, a passive fragment, on the equator. This behaviour is strictly comparable to that found in plants. The more complicated results of having several crossings-over within and proximal to the inversion have been described by Richardson and are shown in the diagram (Text-fig. 33).

The observations are summarised in Table I, p. 492. They show that inversions occur in both *L* and *M* types of chromosomes. Their lengths can be inferred from the length of the bridge and the length of the fragment. They must always be shorter than either of these. Their position in

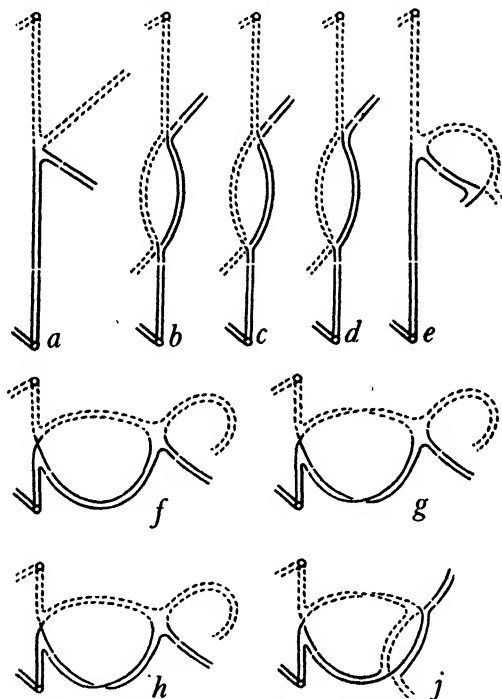


Text-fig. 32. Second "restitution" metaphase in *Chorthippus* 4. A single nucleus has been restored owing to the failure of two *L*-chromosomes to separate, presumably as a result of their having formed two reciprocal chiasmata between relatively inverted segments. $\times 2400$.

the arms of the chromosomes can be inferred from the differences in lengths of bridge and fragment. Thus $\frac{1}{2}(f-b)$ is the amount by which the centre of the inversion is nearer the centromere than to the end of the chromosome. The position thus shown differs for different inversions in the same animal (e.g. in *Chorthippus* 8) and on it depend other differences of behaviour. Thus crossing-over proximal to the inversion can give a bridge at the second division (Text-figs. 31 and 32) and this only occurs when the first division bridge is fairly long, and then less frequently than the first division bridge; in *Stauroderus*, where the chiasma frequency is low, it does not occur at all. As Müntzing found in *Crepis* (1934), the bridge may break in such a way as to leave part of itself lying on the spindle, lost to both nuclei. But the bridge may in other divisions break in the middle (Text-fig. 30 c, d). The bridge may be so short as to be broken early, and

it may then disappear at late anaphase (Text-fig. 30 *b*), or it may be preserved at a second division (Text-fig. 31 *a*).

The fragment varies in its relation to the bridge. It is frequently interlocked with the bridge. This "strangled" fragment, found also by Upcott (unpublished) in *Tulipa*, is held next to the bridge instead of passing to the edge of the spindle as it normally does. In one cell the



Text-fig. 33. Diagram to show the results of crossing-over in a pair of chromosomes heterozygous for a simple inversion not including the centromere (shown by a circle). One chromosome is shown entire, its partner broken. The limits of the inversion are shown by breaks in the entire chromosome. *a*, single crossing-over in the inversion; *b*, reciprocal. *c*, complementary. *d*, disparate. *e*, crossing-over in the inversion and distal to it. *f* to *j*, crossing-over in the inversion and proximal to it. *f*, reciprocal, and *g*, complementary, give first division bridges; *h* and *j* which are disparate with respect to single and double complementary crossing-over give second division bridges.

bridge broke on one side of the strangled fragment, which was consequently dragged by the contracting bridge towards one daughter nucleus (Text-fig. 30 *d*). The strangling of the fragment is presumably the result of the bridge chromatid being coiled round the fragment chromatid. This cannot be due to relational coiling of the cross-over and non-cross-over chromatids. It is to be expected where there has been a loop proximal to the inversion chiasma (Text-fig. 33 *g*, *h*).

Other differences of behaviour depend on the length of the inversion. Most of the inversions are apparently too short to have double cross-overs frequently within their length. Double bridges have not appeared at the first anaphase. But a second division restitution nucleus (Text-fig. 32) apparently resulted from the failure of chromatids from partner chromosomes to separate. They had presumably been held together between two reciprocal chiasmata in the inversion owing to the anomalous direction of the pull in such a configuration (Text-fig. 33 b; cf. Darlington, 1935 c). It seems that in these organisms the anaphase spindle stretches to a sufficient length to break all but the longest of single bridges.

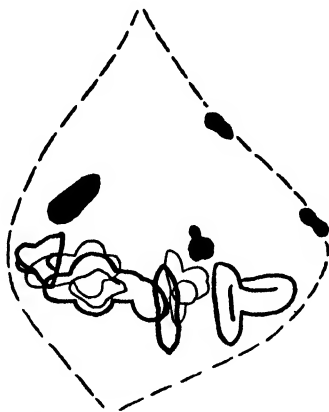
Many earlier descriptions testify to the unrecognized occurrence of bridges resulting from inversion hybridity in different organisms (cf. Richardson, 1936). Belar (1929, Fig. 68) illustrates bridges and describes their breakage in the living cell at first anaphase in *Stenobothrus lineatus*, a species closely related to those described here. He assumed this behaviour to be a consequence of normal chiasma formation.

In several individuals heterozygous for inversions reversed loops appeared at pachytene. These arise from the pairing of corresponding parts of the relatively inverted segments (Text-fig. 28). But they did not appear in all cells, although I have identified the whole complement at this stage in many cells of both species (e.g. Text-fig. 7 represents *Chorthippus* 8, heterozygous for three simple inversions and one translocated inversion). It therefore seems that the relatively inverted segments sometimes pair straight, i.e. non-homologous ends of the inversion come together, as McClintock found in *Zea* (1933).

Such a false pairing must have a serious effect on the frequency of crossing-over in the inversions. Unfortunately this frequency is not determinable with accuracy. Anaphases and later stages showing the results of crossing-over can be satisfactorily studied only in sections in these animals. The chromosomes in a proportion of the cells in sections are cut, so that bridges and fragments cannot be recorded with the necessary precision. I can therefore give no more than rough estimates. All the individuals save one had less than 2 per cent of crossing-over in all their inversions together. The exception, *Chorthippus* 8, had between 5 and 10 per cent; nearly all this was crossing-over in one of its four inversions. And in this individual the behaviour was irregular to a degree that seems significant. Some follicles showed no bridges, while in others about 15 per cent of the anaphases had bridges. The most probable explanation is that where the bridges were absent the inverted segments had undergone false pairing at pachytene. Such behaviour

might well depend on an externally controlled rate of development during polarisation and therefore vary widely from follicle to follicle. This explanation, which agrees with the direct pachytene observations, is made the more probable by the observation that the inverted segments whose length was recorded at pachytene were much longer than the frequency of crossing-over would lead one to expect with regular pairing in every nucleus; the false pairing of necessity abolishes crossing-over in the affected segments.

We have seen that homologous parts of chromosomes may be prevented from pairing by delay and premature division in *Fritillaria* (Darlington, 1935). We have also seen that non-homologous parts



Text-fig. 34. First metaphase in *Chorthippus* 3. Three chromosomes unpaired and one of the univalents divided on the edge of the spindle. $\times 2400$.

may undergo a false pairing, when they lie between paired parts, owing merely to torsion. It follows that homologous parts of chromosomes in a non-hybrid must be supposed to undergo false pairing occasionally following their division, just as non-homologous parts do, and that a certain amount of the pairing observed at the pachytene stage may be of this false kind. Why, it may be asked, should such a distinction be made in the case of homologous parts? Because, if the present theory of crossing-over mechanics is correct, association by attraction between two homologous undivided threads will result in crossing-over when they divide; association by torsion between two homologous divided threads or between two non-homologous undivided threads will not. The first is true pachytene pairing, the second false.

It is occasionally found that whole arms of an *L* bivalent in *Chorthippus* have neither chiasmata nor coiling at early diplotene (Text-fig.

26 e). Whether this results from false pairing or not cannot be said. It evidently results from pairing after the uncoiling of the molecular spiral is almost complete.

Reversed loops have been seen in inversion heterozygotes both at pachytene in *Zea* by McClintock (1933) and at the false pachytene in *Drosophila* salivary glands by Koller (1935) and others. These loops are not however characteristic merely of organisms having anaphase bridges and fragments. Inversions including the centromere have no such behaviour (Text-fig. 28). They are in fact devoid of any cytologically observable results of crossing-over, although they will produce gametes with non-observable duplications and deficiencies like other organisms that are merely interchange hybrids. Classification of the *changes* does not therefore correspond with classification of the *hybrids*.

This calls attention to what seems to be a vital distinction between kinds of structural hybrids, a distinction not depending on the method of change but on the results of the hybridity following the change. The type whose behaviour has been chiefly discussed in the past is that in which no dislocation of segments has taken place in relation to the centromere. Anaphase separation then leads to no departure from the ordinary behaviour where every pair of chromatids belongs to one centromere. These hybrids may be described as *eucentric*. The second type, which is characteristically seen in the present inversion hybrids, has segments which are relatively inverted with respect to the centromere. These inevitably produce, by crossing-over, chromatids with two centromeres and with none. Such hybrids are *dyscentric*. The behaviour follows certain uniform rules independently of whether they arise with or without translocation between different chromosomes or between different arms of the same chromosome. And they are not produced by inversion of a segment if this segment includes the centromere. If on the other hand a segment is translocated from one arm of a chromosome to the other without any change in its direction with respect to the ends, that segment is inverted with respect to the centromere and gives a dyscentric hybrid.

(2) *Deficiency hybrids*

Unequal bivalents formed by the pairing of a larger chromosome with a smaller one have been known since 1901 in the Orthoptera. They usually seem to result from deficiency, *i.e.* the loss of a segment. Whether the homozygous deficiency occurs is not known. Since however the heterozygotes occur so generally in both the Tettigidae and the Acridinae, we

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must suppose that the deficient chromosome is transmitted as readily as the entire one, owing to the lost segment being genetically inert. Belar (1929) found that such bivalents and no others were precocious in *Chorthippus*. This I cannot confirm, for like Janssens (1924) I find precocious bivalents which are equal (Text-fig. 20), and furthermore I find several bivalents precocious in different degrees in the same individual (*P*, *M*₇, and *S* described above). More probably the precocity in *Chorthippus* and *Stauroderus* is determined by inertness, which is itself the condition of survival of deficiencies.

The possible inertness of a precocious chromosome reminds us that Heitz (1933 *et al.*) has supposed that all parts which remain condensed

TABLE I

Index of illustrations

	Indi- vidual	Inversions				Unequal bivalents
		Pachytene	Diplotene	First division	Second division	
<i>Chorthippus parallelus</i>	3	—	—	29 <i>a</i>	—	—
	4	28 <i>a-c</i>	—	—	—	—
	5	—	—	—*	31 <i>b (L)</i>	—
	6	28 <i>d</i>	—	29 <i>e</i> *	—	—
	8	—	28 <i>f</i> †	30 <i>a-g</i> ‡ 28 <i>h, j (L)</i>	31 <i>c, d (L)</i> 31 <i>a (M)</i>	—
<i>Stauroderus bicolor</i>	2	—	—	30 <i>h (M)</i>	—	<i>P</i>
	4	—	—	—	—	—
	6	28 <i>e</i>	—	—	—	<i>P</i>
	7	—	—	—	—	<i>P</i>
	7	—	—	(<i>L</i>)‡	—	<i>P</i>

Note: numbered text-figures are 28–31. *P*, precocious chromosome.

* Bridge accompanied by a long fragment.

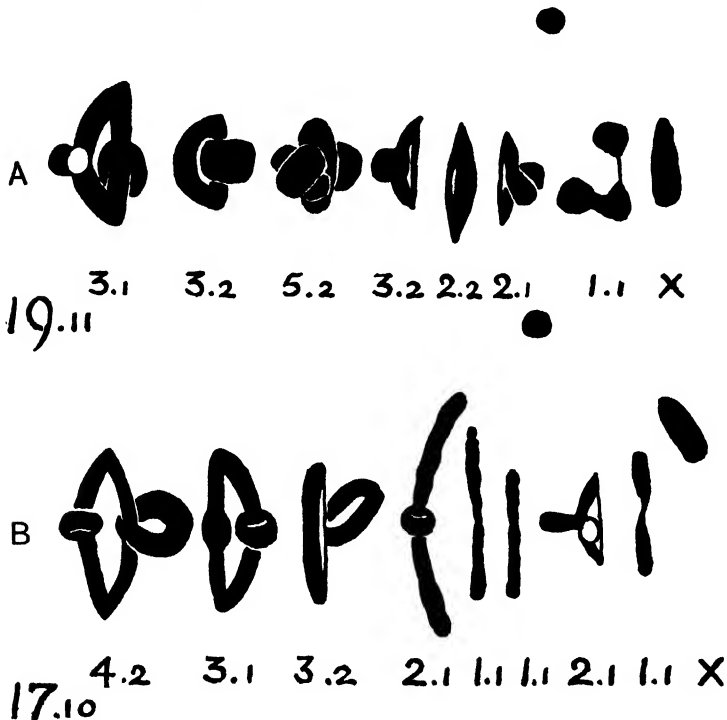
† Inverted translocation to opposite arm of same chromosome.

‡ Seen maintained at second division.

in the resting nucleus of mitosis are inert “heterochromatin”. But there are at least three different kinds of differential condensation: in the resting nucleus in *Pellia* or *Drosophila*, at various stages of meiosis in the heterozygous sex of many animals, and at pachytene in *Agapanthus* and *Fritillaria*. This present evidence of inertness indicates that the first two of these different properties may indeed correspond in other physiological relationships. It seems unlikely on the other hand that the third is to be placed in the same category.

Two individuals of *Stauroderus bicolor* had an unequal bivalent. In one it divided like that in *Phrynotettix magnus* (Wenrich, 1916) either reductionally or equationally at the first division (Text-figs. 35–37). Wenrich attributed this behaviour to the “polarity” of the chromosome

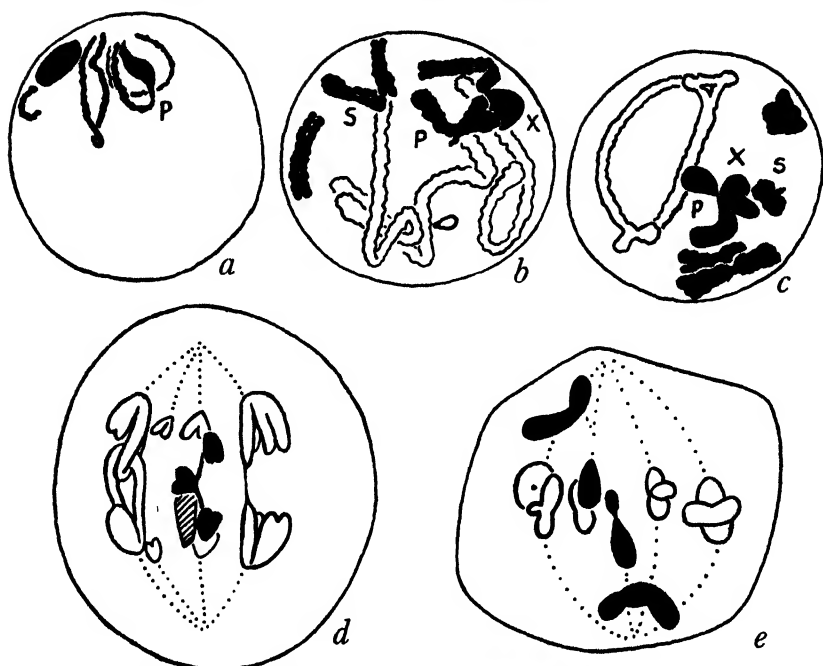
shifting from the equal to the unequal end. I attribute it to crossing-over occurring on either side of a centromere which is intercalary and not terminal. When it occurs on the opposite side to the inequality the first division is reductional in respect of the inequality. When it occurs on the same side, *i.e.* between the centromere and the inequality, the first division is equational (Darlington, 1932 *b*, Fig. 74). This view is made



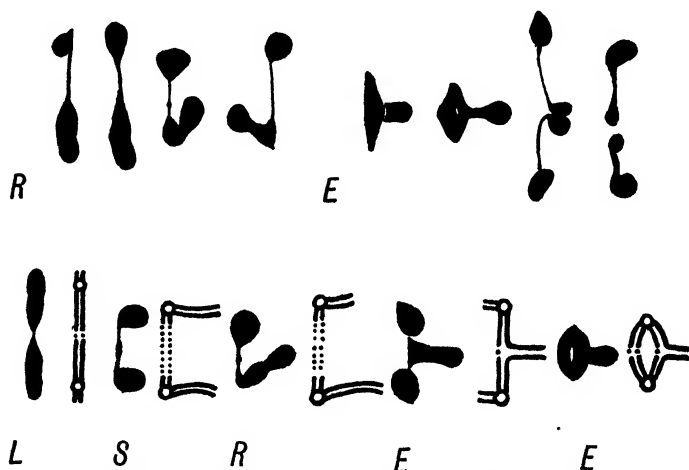
Text-fig. 35. Two first metaphases in *Stauroderus bicolor*. A, normal type but with two *S*-chromosomes unpaired and *P* bivalent unequal. B, slender chromosome nucleus (especially for *M* and *S*). Three *M*-chromosomes have a chiasma in the short arm in A, one in B. Total and terminal chiasmata under each bivalent. $\times 3200$.

possible in *Stauroderus* by the observation that the centromere is not in fact terminal. The types of pairing and segregation are therefore just as described for the sex chromosomes of the rat (Koller and Darlington, 1934).

The different effects of crossing-over and chiasma formation on opposite sides of the centromere may be seen in a simpler way in ordinary short bivalents where the centromere is close to one end and a chiasma in the short arm is consequently rare (Text-fig. 37). Where such a chiasma is



Text-fig. 36. Behaviour of an unequal pair of chromosomes in *Stauroderus*. *c*, No. 7; the rest No. 6. *a* and *b*, early and late pachytene. *c*, diplotene showing that the unequal pair is *P*, the precocious chromosome. *d*, equational separation of the unequal pair. *e*, non-pairing of an *L* pair. $\times 2400$.



Text-fig. 37. Metaphase orientation of bivalents in *Stauroderus* according to the distribution of chiasmata. *R*, to give reductional and *E*, equational separation of unequal bivalents (cf. Darlington, 1932 *b*, Fig. 74). *L* with chiasma in long arm, *S* in short arm, of *M* bivalents. $\times 2400$.

formed it is so close to the centromeres that their mutual repulsion separates this pair of chromosomes earlier than the others. Such precocious separation has been connected by Belar (1929, Fig. 67 *g*, photographs) with the precocious condensation of a bivalent at prophase, but it has no such necessary connection, since it is found wherever this chiasma-centromere relationship exists (*e.g. Secale* fragment, Darlington, 1933).

Conclusion. Nearly all the grasshoppers studied were heterozygous for inversions. The conditions and consequences of crossing-over in them seem to be the same as in plants: the reversed loops at pachytene and the bridges and fragments at anaphase. The positions of the crossings-over in relation to the structural difference determines whether the bridge shall appear at first or second anaphase and whether unequal bivalents shall divide reductionally at first or second anaphase. Three animals were heterozygous for deficiencies in respect of inert segments.

(3) *The hybridity equilibrium*

The occurrence of deficiency hybrids in the population is of no interest beyond implying the inertness of the deficient segment. The occurrence of inversion hybrids on the other hand is of great interest, although its interpretation waits on experimental breeding to test the *genic* neutrality of the inversions. To find that nearly all individuals are inversion hybrids shows what we may call a very high *hybridity equilibrium* within these species. The factors determining this equilibrium are the mutation-frequency, the crossing frequency (between mutant and original types) and the differential survival value of homozygote and heterozygote. What the relative values of these factors may be we cannot yet say. They imply free mating in a large population, rather than a small one; a high rate of structural change rather than a low one; and a low rate of elimination (owing to their slight effect on fertility and their *genic* neutrality) rather than a high one.

It is worth while pointing out how different are the conditions of structural hybridity in cross- and self-fertilised organisms. In plants we have found interchange to be one of the commonest types of change. In animals it seems to be totally excluded. Inversion occurs equally in both, but it will take effect, we may predict, in very different ways in different species, according to the length of the inversions in terms of crossing-over frequency. Long inversions will merely reduce the fertility of F_1 hybrids and thus produce a genetic isolation of individuals, the potential parents.

They will not greatly reduce crossing-over in the inverted segments. Short inversions on the other hand will have the opposite effect. They will have no appreciable effect in reducing the fertility of the hybrid, but they will abolish crossing-over in the inverted segment. They will therefore produce no genetic isolation of individuals but a genetic isolation of segments, a segmental endogamy which will have, as we shall see, an entirely different result in the history of the species.

The importance of the inversions in *Chorthippus* therefore does not depend on the crossing-over in 1 or 2 per cent of cells, by which we so readily identify them, but on the suppression of effective crossing-over in every cell.

The equilibrium attained by short-inversion hybridity within an endogamous group will be higher than for other forms of structural hybridity, since crossing-over will act against inversion only when it occurs within them. The equilibrium will therefore depend on the frequency with which original inversions of different lengths occur, a frequency of which we know nothing at present. Since inversions without crossing-over will be especially selected, these inversions will enjoy segmental endogamy. There must therefore be many shorter inversions with very rare crossing-over in these animals for every one whose crossing-over has revealed its presence. These will all be capable of holding together groups of gene mutations without recombination. As soon as an inversion ceases in this way to be genically neutral it may be eliminated or encouraged by its special gene activity, and this differential effect may operate either on the homozygote or the heterozygote. Effects operating on the homozygote will, if favourable, establish a group-discontinuity, a fission in the species. Effects operating on the heterozygote will raise the hybridity equilibrium if favourable, or determine a fission in the species if unfavourable, having in this case the same eventual effect as a long inversion has directly.

The difference in effect between long and short inversions must be analogous to the difference in effect of interchanges in organisms without and with terminalisation. In the first (like *Zea* and *Pisum*) they produce cumulative sterility of the hybrid, in the second (like *Oenothera* and *Campanula*) they allow the hybrid to replace the homozygote and merely determine group discontinuities by suppressing crossing-over in the differential segments. We never get such a permanent heterozygote in a sexually differentiated species, apart from its sex-determining mechanism, either by inversions or interchanges. But short inversions which are genically neutral can evidently *float* in the species without doing any

harm. If they happen to pick up genes, by their suppression of crossing-over they will become a focus of discontinuity in the species.

We have therefore, it seems, three subjects of study in examining the relation of structural change to species formation: (i) the frequency of different kinds of original change, (ii) the hybridity equilibrium for different kinds of change, (iii) the structural changes distinguishing different endogamous groups (races and species). We have reason to believe that the proportionate frequency of original changes of different kinds, such as inversion and interchange, is similar in the most widely separated organisms. But the hybridity equilibrium is different for the two kinds of change in different groups on account of the different methods of reproduction. While inversions are probably of universal importance, interchanges have never been found in wild populations of animals or as distinctions between animal species. And their function in plants seems to be of two kinds. In some genera they have, like the inversion in *Chorthippus*, a high hybridity equilibrium within the endogamous group. In others they exist chiefly as differences between endogamous groups. The explanation is obvious: in the first the structural difference is floating in the species, relatively innocuous to its bearers. In the second it immediately creates a barrier by the sterility it inflicts on heterozygotes. The first will lead to gradual fission in the species, the second to sudden expulsion from the species.

SUMMARY AND CONCLUSIONS

1. The centric constrictions of all chromosomes in *Chorthippus* and *Stauroderus* are intercalary, as can be seen at mitosis and meiosis. The chromosomes with very short arms occasionally form chiasmata in these arms at meiosis. The lengths of the chromosomes (*i.e.* the degree of spiralisation) vary subject to genotypic and environmental control at mitosis and meiosis. In both genera there are chromosomes wholly or partly precocious in their condensation at meiosis and these are sometimes deficient and therefore perhaps inert.

2. Polarisation at the prophase of meiosis in *Chorthippus* and *Stauroderus* consists in the movement of all the ends of the chromosomes towards a pole. The ends of some of the chromosomes, especially the precociously condensed ones, are attracted towards the X and towards one another. This results in attachment which persists during diplotene. The localised and genotypically controlled staining properties of the condensed chromosomes and the specificity and duration of their attractions are analogous with nucleolar behaviour.

3. During the pachytene stage the chromosomes become relationally coiled. At diplotene this coiling is represented directly, where it has survived, as chromosome and chromatid coiling and also as coiling of the non-cross-over chromatids at the chiasmata, which is consistent with the chromosome coiling. The chromosome coiling and chiasma coiling are lost during diplotene, the chiasmata becoming symmetrical. Chromosome and chiasma coiling is consistent in chromosome arms and opposite in the two arms of long chromosomes. An asymmetrical uncoiling in the four arms of some chiasmata is recognisable, and results from particular spatial relations of the four chromatids at the point of crossing-over.

4. Three individuals of *Stauroderus* were heterozygous for a deficiency in a precocious chromosome.

Eight out of nine individuals of the two species were inversion hybrids with crossing-over in the inversions and in parts proximal to them. The inverted pairing was found at pachytene, and the results of crossing-over at later stages, viz. an inverted chiasma at diplotene and the acentric and dicentric chromatids at anaphase of the first and second meiotic divisions. Direct and indirect evidence shows that relatively inverted segments often pair the wrong way round and then do not cross over. Inversions in the natural population will provide a basis for the origin of group discontinuities, and hence fission in the species, which will be as important with cross-fertilisation as interchange is with self-fertilisation.

POSTSCRIPT

An account of meiosis in various male *Orthoptera* has appeared since the present article was sent to the press (Carlson, March 1936, *J. Morph.* **59**, 123-55). The author disagrees with my previous description of the precocious chromosomes and reaches traditional conclusions in all essential respects.

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EXPLANATION OF PLATE XIII

Microphotographs, $\times ca.$ 2000. Figs. 1, 2 and 3, *Chorthippus* nucleus at diplotene showing association of *X*, *P* and *M*. Figs. 4-6, unequal bivalents. Fig. 4, cf. Text-fig. 35 *A*. Fig. 5, cf. Text-fig. 36 *d*. Fig. 6, cf. Text-fig. 37 *E*. Figs. 7 and 8, an inversion chiasma, cf. Text-fig. 28 *f*. Figs. 9-12, first and second division bridges. Fig. 9, cf. Text-fig. 29 *d*. Fig. 10, cf. Text-fig. 29 *c*. Fig. 11, cf. Text-fig. 30 *f*. Fig. 12, cf. Text-fig. 31 *a*.



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